CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205739Orig1s000

PHARMACOLOGY REVIEW(S)

Reviewer: William T. Link, Ph.D.
10/21/2015
Memorandum re: evaluation of potential for colonic irritancy and/or neoplastic sequelae following chronic use of patiromer

The sponsor, Relypsa, was granted a waiver of carcinogenicity studies based on demonstration that the drug is not absorbed. In the absence of these studies, the potential for colonic irritation, leading to neoplastic pathology with chronic administration, was evaluated based on the longest toxicology studies available, the 26-week study in rats and the 39-week study in dogs. While studies of this duration are not considered adequate to detect slow growing tumors, they are of adequate duration to demonstrate inflammatory responses. The details of these studies are available in the NDA Pharm/Tox review and the original study reports can be found in the electronic submission of NDA 205739.

The rat study is most informative due to the higher exposure (10x relative to the human, vs 7.5x for the dog) and the longer exposure relative to lifespan (25% and 5-10% for the rat and dog, respectively). Additionally, there were more animals in the rat study (30 dosed rats in the high dose group, vs 6 dogs). In the rat study there are no adverse findings or signs of inflammation in the gut.

In the dog study, one dog (1/6) had slightly increased tingible body macrophages in the Peyer's patches of the ileum and colon, an indication of increased lymphocyte turnover. There were no gross observations of inflammation or erosions in ileum or colon reported in this dog.

In this reviewer's opinion, the weight of evidence in these studies suggests that the response of the gut, to the presence of large dose multiples of the drug product patiromer, appears rather benign. If there were colonic irritation, evidence of erosion or ulceration and accompanying inflammation would likely be grossly evident and seen in more of the study animals. It is also a large jump from colonic irritation (which we do not see convincingly) to frank carcinogenesis, and no intermediate stages (polyps, adenomas, etc.) are reported.

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/s/

WILLIAM T LINK
10/21/2015

ALBERT F DEFELICE
10/21/2015

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 205739
Submission date: 10/21/2014
Drug: patiromer
Applicant: Relypsa, Inc.

Indications: hyperkalemia

Reviewing Division: Division of Cardiovascular and Renal Products, Division of Hematology Products

Discussion: The primary and secondary pharmacology/toxicology reviewers found the information for patiromer sufficient to support its approval for the above indication.

Patiromer is a potassium binder which is an existing established pharmacologic class.

Carcinogenicity studies of patiromer were not conducted. The division, in consultation with the executive carcinogenicity assessment committee, decided this was acceptable because the drug is not systemically absorbed, did not produce any systemic toxicity, is not genotoxic and did not produce any preneoplastic effects in the gastrointestinal tract in chronic rodent and non-rodent studies.

A study in rats did not demonstrate any effect on fertility. Embryo fetal studies in rats and rabbits showed no significant fetal effects.

Conclusions: I concur that the nonclinical information is adequate to support approval of patiromer for the indication listed above. I have provided other comments on labeling to the review division separately.

Reference ID: 3836016

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/s/

PAUL C BROWN
10/20/2015

Reviewer: William T. Link, Ph.D.
10/05/2015
Memorandum re: Xanthan gum in patiromer drug product and pediatric use (NDA 205739)

The presence of xanthan gum ((b) (4)%) in preparation of the patiromer drug product has raised concerns for pediatric use in premature infants and the possibility of necrotizing enterocolitis. The underlying mechanism has been postulated to involve irritation due to release of short-chain fatty acids from metabolism of the xanthan gum by intestinal flora. Additionally, investigation of the known instances of necrotizing enterocolitis, associated with use of xanthan gum preparations in premature infants, have not ruled out the possibility of bacterial contamination of the product itself.

In an effort to characterize a safety factor for the total content present in the patiromer preparation ((b) (4) (b) (4) for 0-6 mos age, or (b) (4) for 6-24 mos), the following rationale was used:

Lin, et al. (2002), evaluated the effects of lactic, butyric and acetic acids on the intestinal mucosa of newborn rats. These short chain fatty acids and lactic acid are colonic bacterial fermentation products and represent the primary metabolites expected from xanthan gum breakdown.

Methods

A total of 72 newborn Sprague-Dawley rats (10 days old) were studied. A 3.5F catheter was inserted per rectum 4.0 cm deep into the proximal colon for organic acid administration at a volume of 0.1 ml/10 g body weight. The pH of organic acid solutions and normal saline was adjusted to 4.0. Group 1 (n = 10) received normal saline as a control. Group 2 (n = 11) received 150 mM acetic acid. Group 3 (n = 11) received 300 mM acetic acid. Group 4 (n = 10) received 150 mM butyric acid. Group 5 (n = 11) received 300 mM butyric acid. Group 6 (n = 7) received 150 mM lactic acid, and group 7 (n = 12) received 300 mM lactic acid. Animals were killed 24 hours after colonic installation of test solutions.

Results

Both 300 mM acetic acid and 300 mM butyric acid were associated with impaired weight gain, increased colon wet weight, and increased histologic injury scores in the colon and distal ileum ($P < 0.05$, analysis of variance, see the author's Figure 4 below). Both 150 mM acetic acid and butyric acid at 150 mmol/L induced minimal injury in the colon and distal ileum. Neither 150 mM nor 300 mM lactic acid induced any identifiable gross or microscopic intestinal mucosal injury.

Conclusions

The results, from the 150 mM groups, for butyric and acetic acid suggest that this level is close to a no-effect level. As the dosages were based on body weight, a projected comparable exposure for a one kg infant can be calculated as 900 mg and 1320 mg for acetic acid and butyric acid, respectively.

Therefore, as (b) (4) mg of xanthan gum could produce no more than (b) (4) mg of either of these acids, these results suggest a safety margin of 45 and 66 for acetic and butyric acids, respectively, relative to the exposure that produced minimal effects in newborn rats (Figure 4 below). This ratio is based upon an assumption of complete colonic fermentation of the entire (b) (4) mg of xanthan gum in the patiromer dosage.

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Reference

Jing Lin, Suhas M. Nafday, Sara N. Chauvin, Margret S. Magid, Sudha Pabbatireddy, Ian R. Holzman, and Mark W. Babyatsky. (2002). Variable effects of short chain fatty acids and lactic acid in inducing intestinal mucosal injury in newborn rats. *Journal of Pediatric Gastroenterology and Nutrition,* 35:545–550.

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/s/

WILLIAM T LINK
10/19/2015

ALBERT F DEFELICE
10/19/2015

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:	NDA 205739
Supporting document/s:	SDN 0001
Applicant's letter date:	10/21/14
CDER stamp date:	10/21/14
Product:	VELTASSA™, patiromer, RLY5016S
Indication:	Treatment of hyperkalemia
Applicant:	Relypsa, Inc.
Review Division:	Div. Cardio-Renal Products DCRP
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Albert De Felice, Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Sabry Soukehal

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This application is being submitted for use of RLY5016 for Oral Suspension in the treatment of hyperkalemia. The drug product is formulated as a powder for oral suspension in water administered (b) (4) daily. RLY5016 for Oral Suspension is composed of the drug substance, RLY5016S, formulated with (b) (4) wt% xanthan gum, NF (National Formulary) (b) (4). RLY5016S is a free flowing powder composed of individual spherical beads approximately (b) (4) (b) (4) in diameter. RLY5016S is synthesized from (b) (4) Each polymer bead is (b) (4) that has multiple covalent crosslinks between polymer chains. A calcium-sorbitol (b) (4) counterion to the negatively charged polymer anion.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical pharmacology studies conducted in support of this application used several different forms of the drug substance, in addition to the proposed commercial drug substance, RLY5016S. All contained the identical RLY5016 polymer anion; (b) (4).

Pharmacology

The pharmacology testing of the RLY5016 polymer anion has focused on the primary pharmacodynamic effect of the drug, which is to bind potassium in the lumen of the colon and increase fecal potassium excretion, leading to removal of potassium from the body and lowering of serum potassium levels. The pharmacology studies showed that the RLY5016 polymer anion binds potassium (b) (4) However, RLY5016S is not a specific potassium binder. The RLY5016 polymer anion binds cations (b) (4) The concentration of different cations varies depending on location within the gastrointestinal (GI) tract; as an example, the RLY5016 polymer anion encounters proton and sodium in the upper GI tract where these particular cations are the most abundant. The majority of potassium binding occurs in the colon where potassium is the most abundant cation. By binding potassium in the colon, RLY5016S increases fecal potassium excretion, resulting in a net lowering of serum potassium in the hyperkalemic patient.

Safety pharmacology

Safety pharmacology studies consisted of cardiovascular, central nervous, respiratory and GI systems studies. Pharmacokinetic investigations included

ADME studies in rats and dogs (i.e., the species used to characterize the toxicity profile of RLY5016S), *in vitro* drug-drug interaction studies and an assessment of absorption of fluoride from calcium fluoride (a degradation product of the RLY5016 polymer anion). Given the size of the RLY5016 polymer anion (b) (4) beads), systemic absorption was not expected. The lack of RLY5016 bioavailability was confirmed using (b) (4) RLY5016 beads. Plasma, fecal and urinary monitoring, as well as whole body autoradiography (in rats only), were conducted to assess possible absorption and distribution of the drug.

The oral route of administration was used in the toxicity studies as it was the intended route for use in clinical studies. The highest dose tested was generally limited by the feasibility of administering multiple grams of test article per kilogram of body weight. As agreed with the Agency, carcinogenicity testing and peri/postnatal development studies were not conducted. Toxicity assessments were performed on 34 possible impurities in the drug substance, including the only degradation product, calcium fluoride. These assessments included comprehensive literature searches, *in silico* computational analyses and Ames assays (on seven of the possible impurities). The results of the toxicity assessments were used as part of a systematic and thorough approach to identify, categorize and control impurities in the drug substance.

The primary pharmacodynamics data demonstrate that RLY5016 binds potassium (b) (4) of the mammalian GI tract.

Animal models were used to confirm the mechanism of action of the polymer *in vivo*. These studies showed an increase in fecal potassium excretion in both normal renal function rat and pig models. In addition, a significant decrease in serum potassium was detected in hyperkalemic rats treated with RLY5016, compared to untreated hyperkalemic control animals.

Safety pharmacology studies were conducted to investigate the effects of RLY5016 on the cardiovascular, central nervous, respiratory and GI systems. The maximum recommended human dose of RLY5016 is approximately (b) (4) . Since there is no absorption or metabolism of RLY5016, direct dose comparison (animal/human) can be made to the g/kg NOAEL dosage, determined in safety pharmacology toxicology studies, to establish a safety margin.

Dogs were assigned to a 4 × 4 Latin square for this crossover design study, and each dog received one of four doses (0, 1, 2 or 3.5 g/kg RLY5016) in a predetermined order once weekly. No statistically significant differences were noted for blood pressure (systolic, diastolic, mean arterial pressure and pulse pressure), heart rate or body temperature data. All ECGs were qualitatively and quantitatively within normal limits; no significance (Treatment or Treatment ×

Time) was noted for the QT or QTc data. No arrhythmias were found. No abnormal ECG findings were attributed to the administration of RLY5016.

The nature of the polymeric RLY5016 precluded testing in a hERG assay.

A study of the central nervous system effects of RLY5016, including observations of general behavior, autonomic and motor effects as assessed by the Irwin method, was performed in rats. Single oral RLY5016 doses of 1, 3 and 6 g/kg had no effect in this study (compared to vehicle dosed controls).

The effects of a single oral dose of RLY5016 (0, 1, 3, 6 g/kg) on respiratory parameters (tidal volume, rate of respiration and minute volume) was assessed in freely moving conscious rats using whole body plethysmography. The changes in respiratory parameters observed in the RLY5016-treated groups were not significantly different from the changes observed after vehicle treatment in this study.

Studies were performed to determine if RLY5016 modifies GI transit time in rats (5 per dose) using an orally administered charcoal marker that was administered 1 hour after a single oral dose of RLY5016 (0, 1, 3, 6 g/kg). Twenty-five minutes after the charcoal administration, rats were sacrificed and the distance traveled by the charcoal meal within the small intestine was measured. The stomach and contents were weighed to provide an indication of stomach emptying.

There were no RLY5016-related changes observed on GI transit at 1 and 6 g/kg. However, administration of RLY5016 at a dose level of 3 g/kg produced a slight, but statistically significant, decrease of 23% in GI transit compared to the vehicle group. Administration of RLY5016 at 1 g/kg produced no effect on stomach emptying; however, doses of 3 and 6 g/kg produced a statistically significant decrease in stomach emptying, as demonstrated by an increase in stomach plus contents weight when compared to the vehicle group. RLY5016 at doses of 3 or 6 g/kg produced an increase in stomach plus contents weight of 44%. Given the large doses of RLY5016 administered in this study (approximately 0.5 g in the 3 g/kg dose group and 1 g in the 6 g/kg dose group), residual RLY5016 in the stomach could be contributing to the significant increase (mean increase of 0.8 g) in the stomach plus content weights in these dose groups. Thus, the effects noted on stomach emptying may be physical, as opposed to pharmacological effects.

Toxicology

Rat Studies

Repeat oral dosages as high as 17.6 g/kg/day RLY5016 for 2 weeks (TR 350-07-009), 15 g/kg/day RLY5016 for 4 weeks (TR 350-07-010 and TR 350-07-018) or 5 g/kg/day RLY5016S (dosage expressed in terms of RLY5016) for 26 weeks (TR-350-09-001), administered as a dietary admixture, were well tolerated by

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rats. There were no adverse clinical effects, and there were no toxicologically meaningful changes in clinical pathology (hematology, blood chemistry and urinalysis) or anatomic pathologic evaluations (gross pathology, organ weight and histopathology).

In the 2-week, 4-week and 26-week studies, dosage-related increases in mean food consumption were noted in rats given the higher dosage levels (10 and 15 g/kg/day in the 2-week study; 4, 10 and 15 g/kg/day in the 4-week study and 2.5 and 5 g/kg/day in the 26-week study). These increases in food intake during the dosing period likely reflected the decreased nutritive aspects of the diet (on a weight basis) resulting from the high concentrations of test article in the diet and, as such, were not considered adverse.

In the 4-week study, total serum protein and albumin were significantly lower in high-dosage group female rats and urinary calcium excretion was higher in high-dosage group rats of both sexes. The elevation in urinary calcium excretion was not unexpected since calcium is the counterion associated with the polymer in RLY5016. With the exception of urinary calcium excretion in the high-dosage group females, total serum protein, serum albumin and calcium excretion were similar between control and 15 g/kg/day rats at recovery Day 15. There was no microscopic correlate to these laboratory value differences, and the mean values were similar to control values by recovery Day 15; therefore, the changes were not considered adverse. There were no clinical pathology effects noted in the 26-week chronic toxicity study.

The NOAELs for RLY5016 administered daily in the diet to rats for 2 and 4 weeks was > 17.6 g/kg/day and > 15 g/kg/day, respectively. The NOAEL for RLY5016S administered daily in the diet to rats for 26 weeks was > 5.0 g/kg/day (expressed as RLY5016). No target organ toxicity was identified.

Dog Studies

An initial range-finding study was conducted (TR 350-07-011) in which beagle dogs were administered 7 g/kg/day RLY5016 for 10 days, twice daily, in gelatin capsules. The number of capsules was found to limit the amount of drug that could be administered to dogs. Therefore, a maximum feasible dosage of 7 g/kg/day was reached in the study. The maximum tolerated dosage of RLY5016 in this study was determined to be > 7 g/kg/day.

In the longer term repeat-dose studies in dogs, oral (gelatin capsule) dosages as high as 7 g/kg/day RLY5016 for 4 weeks (TR 350-07-015 and TR 350-07-019) or 3.75 g/kg/day RLY5016S (dose expressed in terms of RLY5016) for 39 weeks (TR 350-10-001), were well tolerated by dogs. There were no adverse clinical effects, and there were no toxicologically meaningful changes in clinical pathology (hematology, blood chemistry and urinalysis), anatomic pathologic evaluations (gross pathology, organ weight and histopathology), ophthalmic or ECG parameters.

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In the 39-week study, significantly lower mean food consumption occurred in the high-dosage group females during Weeks 2, 4, 14, 15, 19 and 22 with no adverse impact on mean body weight or body weight change. Vomiting of capsules occurred infrequently with no adverse impact on RLY5016S dosing.

The NOAEL for RLY5016 administered daily to dogs for 4 weeks was > 7 g/kg/day; the NOAEL for RLY5016S administered daily to dogs for 39 weeks was determined to be > 3.75 g/kg/day (expressed as RLY5016). No target organ toxicity was identified.

Genotoxicity

The evaluation of the genotoxic potential of RLY5016S was conducted instandardized battery of *in vitro* and *in vivo* tests.

RLY5016 was tested in the in vitro bacterial reverse mutation assay (Ames assay) (TR 350-07-012). In the main study, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvr*A were used as tester strains, and the RLY5016 dose levels ranged from 33.3 – 5,000 microgram/plate; the highest dose being the maximum ICH recommended concentration (ICH S2A). The main study was conducted in the presence and absence of microsomal enzymes derived from Aroclor-induced rat liver (S9) mix. Based on the results, RLY5016 was not mutagenic in the *in vitro* bacterial mutation assay.

The ability of RLY5016 to induce chromosome aberrations was evaluated in cultured Chinese hamster ovary (CHO) cells. The study was conducted in two phases: an initial assay and a confirmatory assay (TR 350-07-013). Based on the results from the initial assay, the confirmatory chromosomal aberration assay was conducted at RLY5016 doses of 15.6 – 500 micrograms/mL without metabolic activation and 31.3 – 500 micrograms/mL with metabolic activation. No significant increase in cells with chromosomal aberrations, polyploidy or endoreduplication was observed. Thus, RLY5016 was negative for inducing structural chromosomal aberrations in CHO cells with and without metabolic activation.

RLY5016 was evaluated for clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocytes (PCEs) in rat bone marrow (TR 350-07-014, titled "*In Vivo* Rat Bone Marrow Micronucleus Assay with RLY5016"). Rats were administered a single dose of RLY5016 at dosages of 0, 1, 3 and 6 g/kg via oral gavage. The frequency of micronucleated bone marrow PCEs was assessed at 24 hours (all dose groups) and at 48 hours (high dose group) after dosing. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 total erythrocytes for each animal. RLY5016, administered at dosages up to 6 g/kg,

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was not cytotoxic to the bone marrow and did not induce statistically significant increases in micronucleated PCEs. Thus, RLY5016 was non-mutagenic in the *in vivo* bone marrow micronucleus test.

Carcinogenicity
The requirement for carcinogenicity testing of RLY5016S was waived by the Division

Reproductive Toxicity
The reproductive and developmental toxicity of RLY5016S was evaluated in a rat fertility study, and rat and rabbit teratology studies at maximum feasible dosages. No effects, outside of nutritionally-related effects on the dams and offspring, were noted at any treatment level, and the NOAEL was the highest dose used. These results are as expected, given the lack of systemic exposure of this drug.

As agreed with the Division (Minutes of End-of-Phase 2 Meeting, 22 November 2011), a peri/postnatal development study of RLY5016S was not conducted. There have also been no studies involving the treatment of juvenile animals.

1.3 Recommendations

1.3.1 Approvability

The preclinical toxicology program was well conducted and thorough. The studies were well planned and employed sufficient numbers of dosage groups and animals to allow proper interpretation and review.

The sponsor clearly demonstrated that the compound is not absorbed from the gut, binds potassium in the colon, and results in a lowering of serum potassium.

Toxicity studies demonstrated that the compound is inert and non-toxic. The potential for drug-drug interactions were sufficiently characterized.

This reviewer agrees with the sponsor's interpretation of the toxicity data, as presented, and recommends approval of RLY5016 for the indication sought.

1.3.2 Additional Non Clinical Recommendations

none

1.3.3 Labeling
Labelling review was conducted independently of this document. No major changes to the label were recommended.

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2 Drug Information

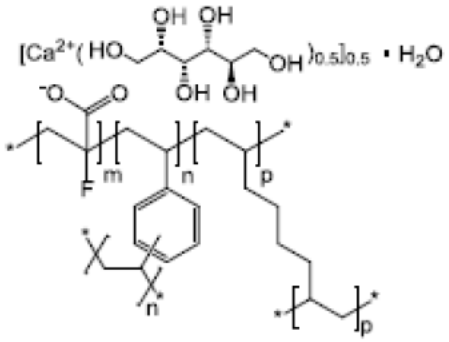
2.1 Drug

CAS Registry Number: 1415477-49-4

Code Name: RLY5016S (patiromer calcium-[redacted])

Chemical Name: [redacted]

Structure or Biochemical Description:



m = number of 2-fluoro-2-propenoate groups m = 0.91

n, p = number of crosslinking groups n + p = 0.09

' H_2O = associated water

* = indicates an extended polymeric network

Molecular Formula

[redacted]

Empirical Formula
RLY5016S: $C_{613}H_{765}F_{114}O_{399}Ca_{57}$

Molecular Weight
Each RLY5016S crosslinked [redacted] bead represents [redacted].
Molecular weight of a [redacted] RLY5016S bead: [redacted]

Pharmacologic Class: Potassium binding resin

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 75615

2.3 Drug Formulation

The drug substance, RLY5016S, is an amorphous, free flowing powder that is composed of individual spherical beads. The intended commercial formulation (Formulation C) for the drug product is composed of RLY5016S and xanthan gum. RLY5016 for Oral Suspension is intended to be administered orally after suspending in water.

2.4 Comments on Novel Excipients

none

2.5 Comments on Impurities/Degradants of Concern

none

2.6 Proposed Clinical Population and Dosing Regimen

VELTASSA™ (patiromer) is indicated for the treatment of hyperkalemia. The recommended starting dose of VELTASSA is (b) (4) 8.4 grams patiromer (b) (4) with meals, based on serum potassium level.

3 Studies Submitted

3.1 Studies Reviewed

All GLP toxicology studies (general, genetic and reproductive) and safety pharmacology studies were reviewed previously under IND 75615, or reviewed herein.

3.2 Studies Not Reviewed

Pharmacology and pharmacokinetic studies are presented as submitted by the sponsor, but were not formally reviewed.

3.3 Previous Reviews Referenced

IND 75615 original IND (SDN 000), also Serial #40 (rec'd 3/8/12)

4 Pharmacology

Nonclinical pharmacology studies conducted in support of this NDA used two different forms of the drug substance, RLY5016 and RLY5016H. The test articles RLY5016 and RLY5016H [redacted (b) (4)]. Each of these test articles contains the polymer anion, which is the active moiety of RLY5016S.

Some of the nonclinical pharmacology studies described in this section expressed dose in terms of RLY5016 (calcium salt of the polymer anion); [redacted (b) (4)]

The nonclinical pharmacology studies described in this section include primary pharmacodynamics and safety pharmacology studies. No secondary pharmacodynamics studies were conducted.

Several of the studies described in this section were conducted and/or sponsored by the previous sponsor, Ilypsa, Inc. (Santa Clara, CA). The development rights to RLY5016 were transferred to Relypsa, Inc., in October 2007. The reports refer to RLY5016 by the code number ILY105; however, this compound is [redacted (b) (4)] RLY5016.

4.1 Primary Pharmacology

4.1.1 Introduction

The primary pharmacology data demonstrate that RLY5016 binds potassium [redacted (b) (4)] *In vitro* studies were conducted to assess the extent of calcium ion exchange and the potassium binding capacity of RLY5016 under conditions that mimic selected compartments of the human GI tract. In the first *in vitro* study, exposing RLY5016 to an acidic environment that mimics conditions in the fasted stomach demonstrated that calcium is exchanged for protons, providing evidence for the fundamental ion exchange mechanism of the polymer. A second *in vitro* study showed that 8.5 – 8.8 mEq of potassium are bound per gram of polymer at a physiologically relevant pH (pH 6.5). Since the potassium binding capacity of the polymer under highly basic conditions (100 mM potassium hydroxide [KOH], pH 12) was 8.4 – 10 mEq/g (corresponding to the maximum binding capacity of the polymer), these data suggest that ≥ [redacted (b) (4)]% of the total binding capacity of the polymer is available for cation exchange under physiological conditions (pH 6.5).

Animal models were used to confirm the mechanism of action of the polymer *in vivo*. These studies showed an increase in fecal potassium excretion in both normal renal function rat and pig models. In addition, a significant decrease in serum potassium was detected in hyperkalemic rats treated with RLY5016, compared to untreated hyperkalemic control animals.

The RLY5016 polymer encounters a series of distinct cation environments as it traverses the GI tract, marked by fluctuations in proton, sodium, potassium, calcium and magnesium concentrations (Fordtran, 1966, Wrong, 1965). The RLY5016 polymer functions [redacted] (b) (4); variations in the cation environment of the GI tract manifest as differences in the cations bound to the polymer. Two *in vitro* pharmacology studies were conducted to assess the extent of calcium counterion exchange and the potassium binding capacity of the polymer under conditions that mimic the stomach and small intestine.

4.1.2 *In vitro* Pharmacodynamics

4.1.2.1 RLY5016 Calcium Cation Exchange (TR 300-07-026)
This study was designed to assess the potential for calcium to exchange with another cation (i.e., proton). RLY5016 was exposed to USP Simulated Gastric Fluid without Pepsin [SGF], pH 1.2 at concentrations of 1, 2, 5, 10 and 20 mg/mL, and free cation concentrations were determined after incubation at 37°C for 2 hours. Subsequently, experiments were repeated for the 5, 10 and 20 mg/mL RLY5016 concentrations. In addition to calcium, sodium was measured in these experiments because it is a component of SGF that can bind to the RLY5016 polymer. The extent of sodium binding was much lower than proton binding, since protons are the predominant cation in the exchange buffer. Experimental results are summarized in Table 1.

Table 1: RLY5016 Polymer Ionic Exchange in an Acidic Environment

RLY5016 Concentration (mg/mL)	Calcium Released[a] (mEq/g) (Mean ± SD)	Sodium Bound[b] (mEq/g) (Mean ± SD)	Final pH (Mean ± SD)
Experiment 1 (N = 3)			
1	7.31 ± 0.33	0.01 ± 0.04	1.25 ± 0.01
2	7.08 ± 0.08	0.04 ± 0.02	1.28 ± 0.02
5	7.20 ± 0.05	0.06 ± 0.00	1.40 ± 0.01
10	6.84 ± 0.02	0.08 ± 0.01	1.83 ± 0.05
20	4.14 ± 0.02	0.1 ± 0.01	2.70 ± 0.01
Experiment 2 (N = 3)			
5	7.31 ± 0.11	0.03 ± 0.01	1.42 ± 0.02
10	6.97 ± 0.02	0.07 ± 0.01	1.86 ± 0.00
20	4.19 ± 0.01	0.1 ± 0.00	2.68 ± 0.01

[a] Calcium release (mEq/g) was calculated as $2 \times Ca_{eq} \times$ dilution factor/RLY5016 concentration, where Ca_{eq} is the calcium concentration (mM) measured in the supernatant samples.

[b] Sodium binding (mEq/g) was calculated as $(Na_{start} - Na_{eq}) \times$ dilution factor/RLY5016 concentration, where Na_{start} is the sodium ion concentration (mM) measured in the diluted control samples and Na_{eq} is the sodium ion concentration (mM) in the diluted supernatant samples after incubation with RLY5016.

The lot of RLY5016 used in these experiments (Lot No. PAX1636Ca) had a measured calcium content of (b) (4) wt%, which is equivalent to (b) (4) of calcium per gram of polymer. Results obtained in these experiments indicate that calcium exchange from RLY5016 in SGF was more than (b) (4)% complete for polymer concentrations of 1, 2 and 5 mg/mL (corresponding to (b) (4) of calcium released, respectively). When higher concentrations of polymer were tested, a smaller proportion of the calcium was exchanged from the polymer (e.g., at 10 mg/mL RLY5016, approximately (b) (4)% of the bound calcium was replaced and at 20 mg/mL RLY5016, approximately (b) (4)% of the calcium was replaced). In all cases, very little sodium binding was noted, indicating that calcium release is driven by exchange with proton and not sodium. As the test polymer concentration was increased, the total amount of calcium released into the test solution increased and more proton was bound by the polymer, resulting in an increase in the pH of the test solution. The elevated pH and increased calcium concentration in the test solution both limit the extent of calcium exchange at the highest polymer concentration.

In conclusion, this *in vitro* study indicates that calcium bound to the RLY5016 polymer can be exchanged for proton, providing evidence for the fundamental ion exchange mechanism of the polymer.

4.1.2.2 RLY5016 Potassium Binding (TR 300-07-025)

As described in the preceding section, RLY5016 is a calcium-loaded polymer that exchanges a portion of the calcium counterion for cations such as proton or potassium that represent major cation species in the gastric and colonic environments, respectively. In order to measure the potassium binding capacity of the polymer *in vitro* under a pH condition (Fallingborg, 1989) and a residence time (Rao, 2009, Read, 1980) characteristic of the large intestine, binding experiments were conducted using the protonated polymer (RLY5016H; 4 mg/mL) incubated for 24 hours at room temperature in a simple potassium buffer (200 mM 2-morpholinoethanesulfonic acid [MES], 150 mM K_+) at pH 6.5. To synthesize the protonated polymer, the calcium counterion of RLY5016 was exchanged for proton by (b) (4) The results from two replicate experiments showed no residual calcium in the supernatant, indicating that the ion exchange process (calcium for proton) was complete. Comparative experiments were conducted using other cation exchange resins, including the proton form of poly(methacrylic acid) (Amberlite IRP-64H) and polystyrene sulfonate (Dowex 50WX4 and Kayexalate H) resins. Experimental results are summarized in Table 2.

Table 2: Potassium Binding by Protonated Polymers under Conditions Mimicking Large Intestine pH and Residence Time

Polymer (4 mg/mL)	Potassium Bound[a] (mEq/g) (Mean ± SD)	Final pH[b] (Mean ± SD)
Experiment 1 (N = 3)		
RLY5016H	8.53 ± 0.13	6.21 ± 0.02
Amberlite IRP-64H	3.62 ± 0.28	6.42 ± 0.02
Kayexalate H	5.09 ± 0.05	6.35 ± 0.02
Dowex 50WX4	5.25 ± 0.11	6.34 ± 0.02
Experiment 2 (N = 3)		
RLY5016H	8.77 ± 0.35	6.23 ± 0.01
Amberlite IRP-64H	3.75 ± 0.05	6.43 ± 0.02
Kayexalate H	4.8 ± 0.3	6.36 ± 0.01
Dowex 50WX4	4.97 ± 0.03	6.35 ± 0.02

[a] Potassium (K) bound to the test polymers (mEq/g) was calculated as (K_{start} - K_{eq}) × dilution factor/polymer concentration, where K_{start} is the potassium ion concentration (mM) measured in the diluted control sample and K_{eq} is the potassium ion concentration (mM) in the diluted test samples after incubation with polymer.

[b] Starting pH was 6.5.

The data from this *in vitro* study demonstrate that RLY5016H binds 8.5 – 8.8 mEq of potassium per gram of polymer at physiologically relevant pH (pH 6.5). The potassium binding capacity for this polymer under highly basic conditions (100 mM KOH, pH 12) that produce the maximum binding capacity, was 8.4 – 10 mEq/g. Therefore, at physiological pH, (b) (4)% of the total binding capacity of the polymer is available for cation exchange.

Amberlite IRP-64H, a poly(methacrylic acid) resin, had a total capacity of 10.4 mEq/g under basic conditions (100 mM KOH, pH 12), however it bound only 3.6 – 3.8 mEq of potassium per gram of resin at physiological pH, corresponding to (b) (4)% of the total binding capacity for the polymer. Kayexalate H and Dowex 50WX4 resins contain strongly acidic sulfonate groups, which are fully ionized in the physiological pH range and use their full capacity to bind potassium under these conditions. However, the total capacity of these two resins in proton form is only 4.8 – 5.3 mEq/g. Although all the tested polymers were in proton form at the beginning of the experiment, their final state is one of equilibrium between the polymers and an aqueous medium containing a particular potassium concentration at a physiological pH. The ability of RLY5016H to bind potassium at physiological pH is an inherent property of the polymer, and is not dependent on starting with the acid form.

4.1.3 *In Vivo* Pharmacodynamics

The *in vivo* pharmacodynamic effects of RLY5016 potassium binding were evaluated in three animal models: rats and pigs with normal renal function (TR 300-07-009 and TR 300-07-010, respectively) and rats with chronic renal failure (TR 300-07-027). These models all demonstrated the ability of RLY5016 to promote an increase in fecal potassium excretion, along with increases in the excretion of other cations. In addition, the rat chronic renal failure model demonstrated a reduction in elevated serum potassium in the treated animals.

4.1.3.1 Pharmacologic Effects of RLY5016 in Rats with Normal Renal Function (TR 300-07-009)

The cation binding properties of RLY5016 were evaluated *in vivo* in Sprague-Dawley rats with normal renal function. Rats (six per group) housed in metabolic cages were fed a low calcium diet supplemented with RLY5016 at a daily dose of approximately 0, 0.8 or 1.8 g/kg/day for 7 days. Urine and feces from Day -1 and Days 3 − 6 (24-hour collections) were analyzed for cation content. Body weights were measured on Days -1, 4 and 7. On Day 7, animals were euthanized and blood was collected via cardiac puncture.

After 7 days of treatment with RLY5016, significant cation electrolyte changes were noted in fecal (Figure 1), urine (Figure 2) and serum (Figure 3) samples, relative to untreated controls. In all cases, significant changes were only detected in the 1.8 g/kg/day dose group, with the exception of fecal calcium measured in the 0.8 g/kg/day dose group. Group mean data are provided in Table 3 (fecal and urine electrolytes) and Table 4 (serum electrolytes).

Analysis of fecal cations in rats with normal renal function treated with 1.8 g/kg/day RLY5016 revealed a significant 2.5-fold increase in potassium content of the fecal extracts compared to untreated control animals. Similarly, significant increases in sodium (6.9-fold), magnesium (1.3-fold) and calcium (9.6-fold) were also noted in the fecal extracts prepared from the 1.8 g/kg/day dose group. Substantial increases in these cations were not detected in the low dose (0.8 g/kg/day) group, with the exception of calcium (3.9-fold increase compared to untreated controls). Given the activity of RLY5016 as an ion exchange polymer, the increase in fecal cation content is consistent with cation binding by the polymer supplementing normal fecal cation excretion. It is believed that an increase in fecal calcium occurred because much of the calcium of RLY5016 was either not exchanged or, if exchanged, was not absorbed.

Analysis of urinary cations in rats with normal renal function treated with 1.8 g/kg/day RLY5016 revealed a significant increase (2-fold) in urinary calcium and a significant decrease (35%) in urinary magnesium; however, these changes were not seen in the low dose (0.8 g/kg/day) group. There were no changes in urinary potassium or sodium in either highor low-dose RLY5016 groups. The increase in urinary calcium is expected from the absorption of some of the

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calcium exchanged from the polymer for other cations as the resin traverses the GI tract of the test animals. RLY5016 has an affinity for divalent cations, such as magnesium, that could lead to a transient decrease in urinary magnesium content such as that observed in the rats in this study.

Analysis of serum cation content after treatment with RLY5016 demonstrated a significant reduction in serum potassium and serum magnesium in the high, but not the low, dosage group compared to untreated controls. No changes were noted in serum sodium or serum calcium. In rats with normal renal function, fecal potassium was increased, but no compensatory decrease in urinary potassium was observed, perhaps explaining the net decrease in serum potassium noted in the high dosage group. In this rat study, a small but significant increase in fecal magnesium was noted, accompanied by a decrease in urinary magnesium, but the balance between these two processes was apparently not sufficient to prevent an overall decrease in serum magnesium.

Assessment of food consumption showed that rats in the high dose group consumed significantly more chow on Day 4 only. No differences were observed in mean body weights between treatment groups throughout the study; all animals gained weight and showed no signs of illness or malnourishment.

In conclusion, this study demonstrated that RLY5016 was an effective potassium binder in the GI tract of rats.

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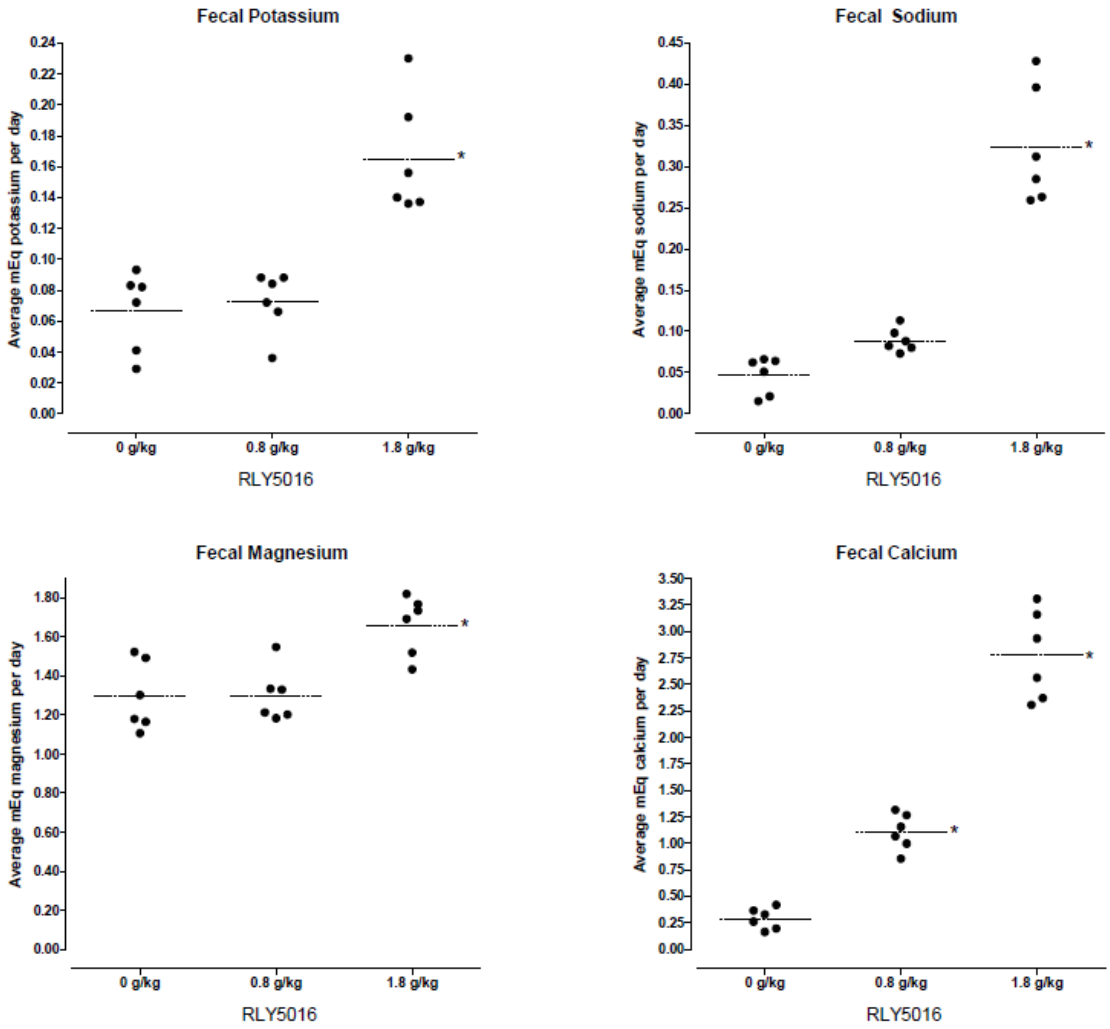
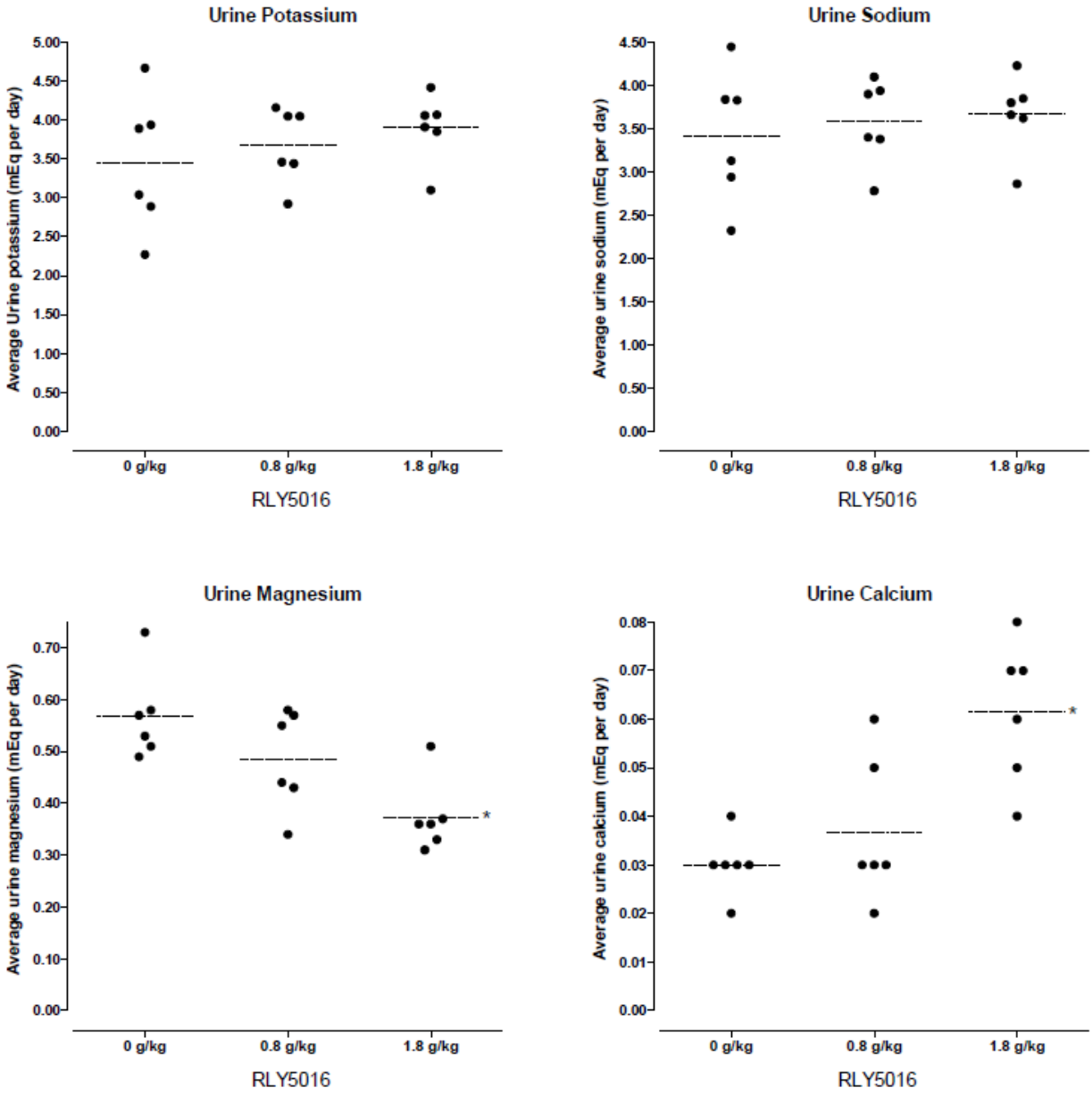
Figure 1: Fecal Electrolytes in RLY5016-treated Rats with Normal Renal Function



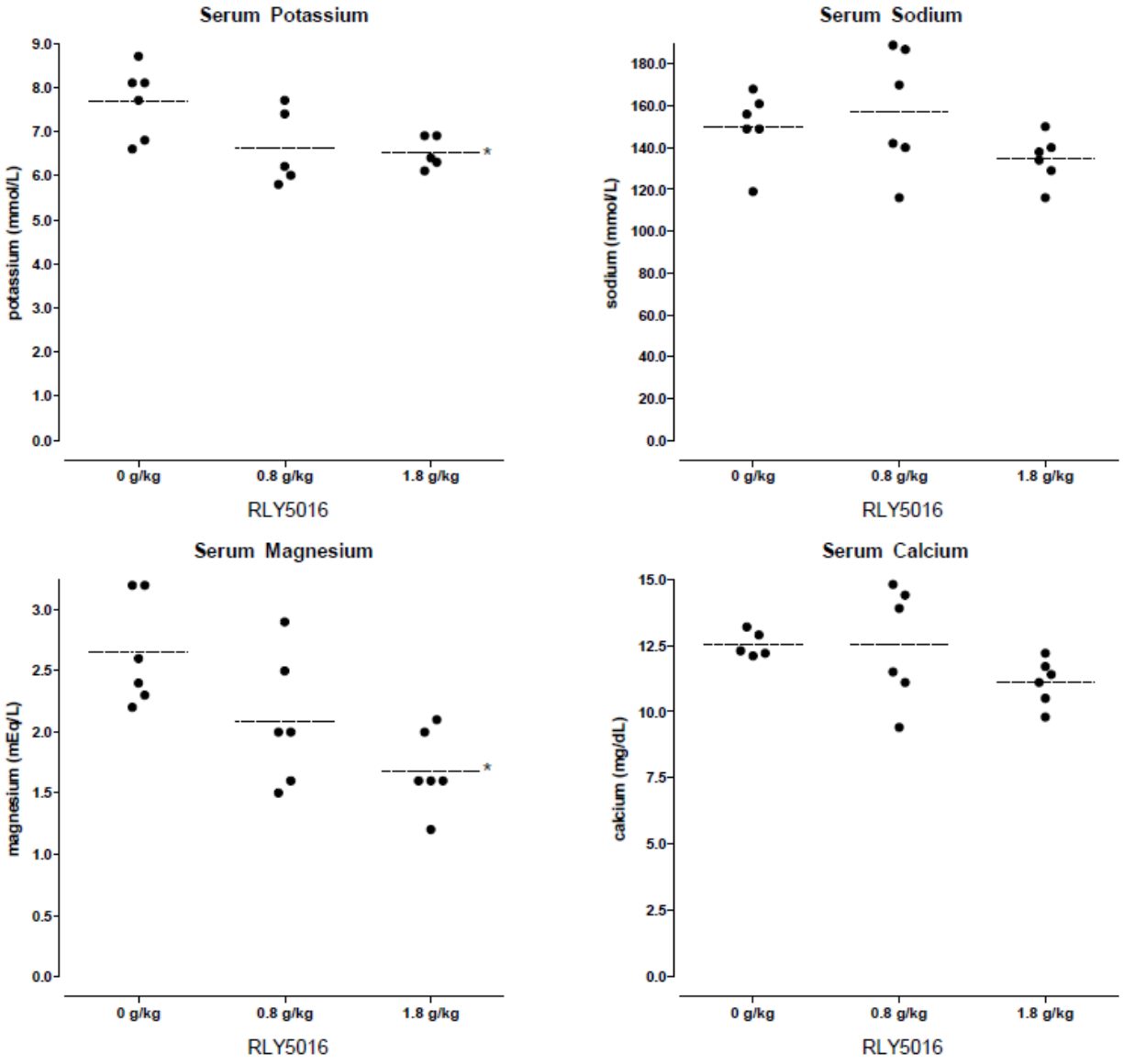
Each symbol represents the average daily value for one rat from Days 3 – 6, and the horizontal bar represents the mean value for the group. Groups whose mean values were significantly different from the non-treated group are indicated with an asterisk ($p < 0.01$).

Figure 2: Urine Electrolytes in RLY5016-treated Rats with Normal Renal Function



Each symbol represents the average daily value for one rat from Days 3 – 6, and the horizontal bar represents the mean value for the group. Groups whose mean values were significantly different from the non-treated group are indicated with an asterisk ($p < 0.01$).

Figure 3: Serum Electrolytes in RLY5016-treated Rats with Normal Renal Function



Each symbol represents the value for one rat on Day 7, and the horizontal bar represents the mean value for the group. Groups whose mean values were significantly different from the non-treated group are indicated with an asterisk ($p < 0.05$).

Table 3: Fecal and Urine Electrolytes after 7 Days of Treatment with RLY5016

RLY5016 (g/kg/day) (N = 6 per dose group)	Potassium (mEq/day) (Mean ± SD)	Sodium (mEq/day) (Mean ± SD)	Magnesium (mEq/day) (Mean ± SD)	Calcium (mEq/day) (Mean ± SD)
Fecal Electrolytes				
0	0.067 ± 0.026	0.047 ± 0.023	1.296 ± 0.177	0.288 ± 0.099
0.8	0.072 ± 0.020	0.089 ± 0.015	1.302 ± 0.137	1.109 ± 0.172
1.8	0.165 ± 0.038	0.324 ± 0.072	1.662 ± 0.151	2.774 ± 0.421
Urine Electrolytes				
0	3.452 ± 0.869	3.418 ± 0.767	0.567 ± 0.085	0.030 ± 0.006
0.8	3.680 ± 0.486	3.582 ± 0.493	0.484 ± 0.097	0.037 ± 0.015
1.8	3.901 ± 0.441	3.673 ± 0.452	0.373 ± 0.069	0.060 ± 0.016

Table 4: Serum Electrolytes after 7 Days of Treatment with RLY5016

RLY5016 (g/kg/day) (N = 6 per dose group)	Potassium (mmol/L) (Mean ± SD)	Sodium (mmol/L) (Mean ± SD)	Magnesium (mEq/L) (Mean ± SD)	Calcium (mg/dL) (Mean ± SD)
0	7.67 ± 0.82	150.33 ± 16.99	2.65 ± 0.45	12.54 ± 0.48[a]
0.8	6.62 ± 0.87[a]	157.33 ± 29.28	2.08 ± 0.53	12.52 ± 2.16
1.8	6.52 ± 0.36[a]	134.50 ± 11.45	1.68 ± 0.33	11.12 ± 0.86

[a] N = 5 samples

4.1.3.2 Pharmacologic Effect of RLY5016 in Normal Renal Function Pigs (TR 300-07-010)

Because pigs provide a good model of the human GI system, (Jensen, 1996, Stevens, 1977), RLY5016 was evaluated in pigs with normal renal function to assess the pharmacological effects of the polymer in binding and removing potassium. RLY5016 was fed to a group of seven pigs for 10 days at a dose of 1 g/kg/day as a diet admixture; a group of seven control pigs were maintained on chow alone. Twenty-four (24)-hour urine collection was performed from Days 1 – 9 and urine electrolytes (potassium, calcium, sodium and magnesium) were assessed. The GI transit time, determined with a ferric oxide marker in the pigs used in this experiment, was < 60 hours; therefore, fecal cation content was assessed in 24-hour fecal samples collected from Days 3 – 8. Serum electrolytes were determined on Day 10 through the analysis of blood samples collected via jugular venipuncture from each pig.

Pigs fed a diet supplemented with 1 g/kg/day of RLY5016 showed a significant increase in potassium, sodium and calcium levels when fecal extracts of the treated group were compared to those of the untreated group (Figure 4 and Table 5).

In contrast, the RLY5016-treated pigs had significantly less urinary potassium and sodium excretion than the non-treatment group (Figure 5 and Table 5). This result is expected in animals with normal renal function, where increases in fecal potassium and sodium excretion are balanced by compensatory decreases in urine elimination of the cations. As was observed in rats with normal renal function (TR 300-07-009; summarized in Section 2.6.2.2.2.1), the RLY5016-treated pigs also had significantly more urinary calcium excretion than the non-treatment group ($p < 0.0001$).

No significant differences were observed between the treatment and non-treatment groups for serum potassium, sodium, magnesium or calcium levels (Figure 6 and Table 6), reflecting compensatory changes in excretion of these cations by fecal and urinary routes in animals with normal renal function.

In conclusion, this study demonstrated that RLY5016 effectively increases fecal potassium excretion and decreases urinary potassium excretion in pigs with normal renal function. These results are consistent with the mechanism of action of the drug as a potassium binder in the mammalian GI tract.

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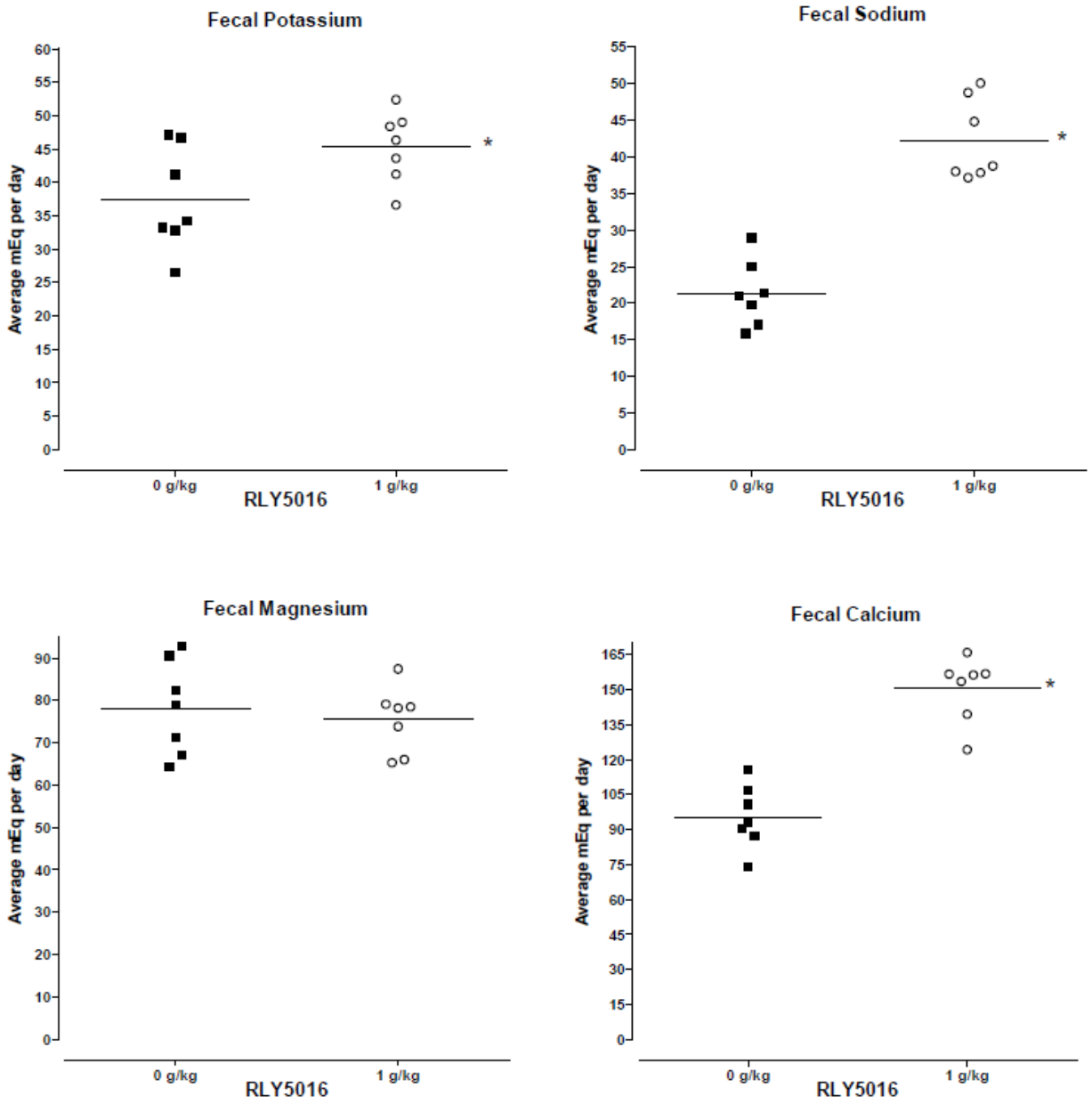
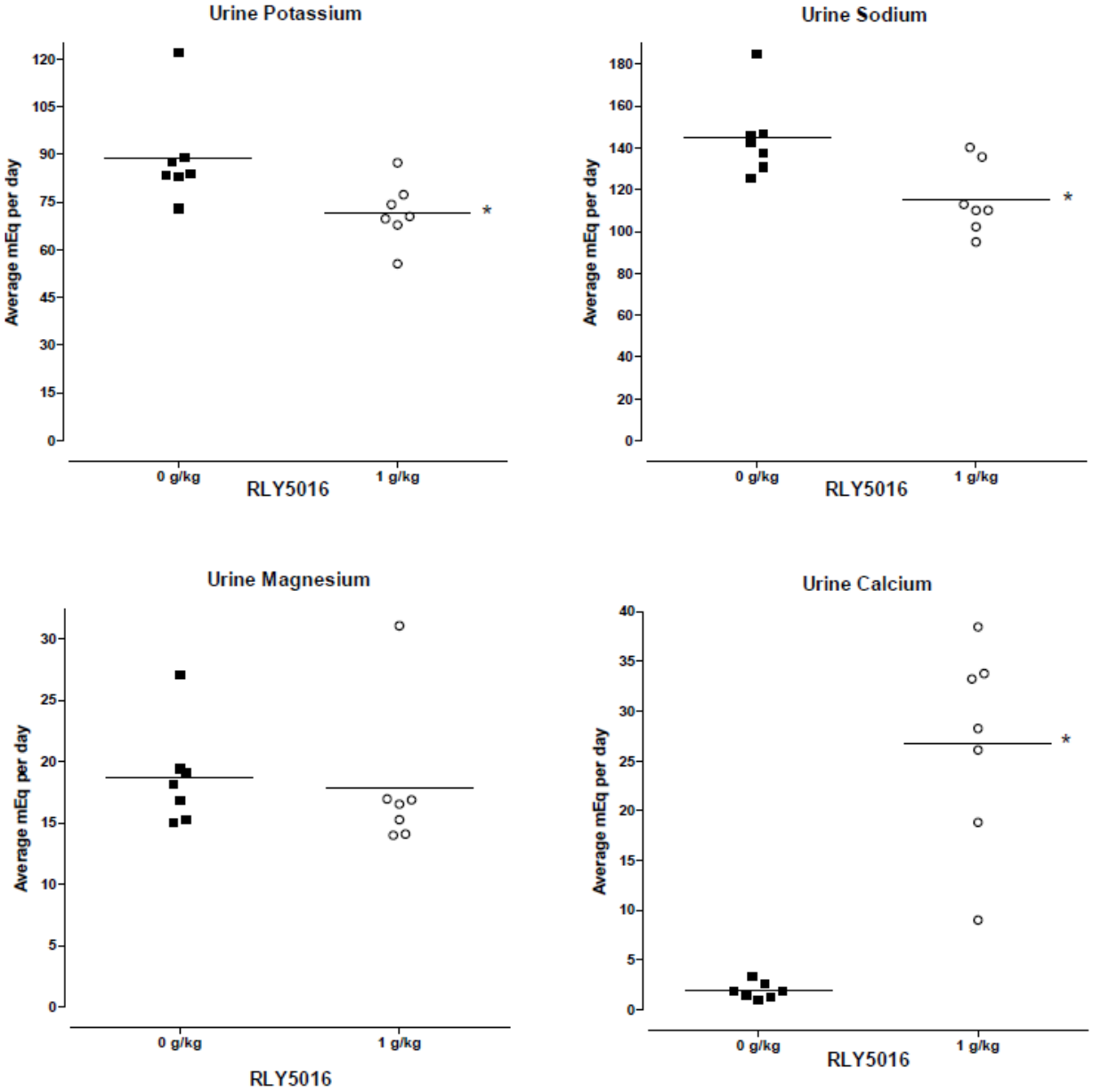
Figure 4: Fecal Electrolytes in RLY5016-treated Pigs with Normal Renal Function



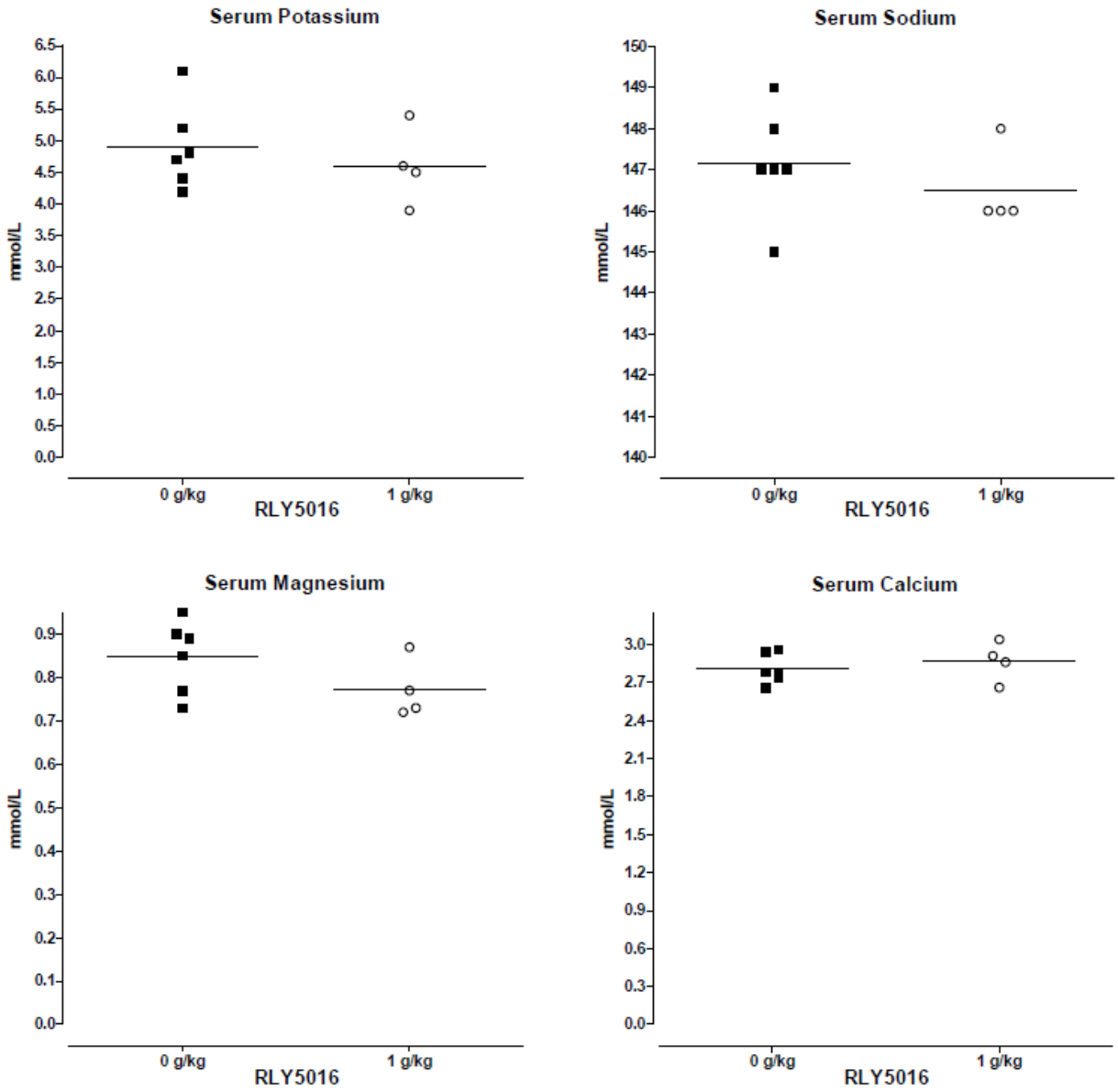
Symbols represent the average daily value from the period Day 3 – Day 8 for each individual animal. The horizontal bar represents the group mean. Statistical significance is indicated by an asterisk ($p < 0.05$).

Figure 5: Urine Electrolytes in RLY5016-treated Pigs with Normal Renal Function



Symbols represent the average daily value from the period Day 1 – Day 8 for each individual animal. The horizontal bar represents the group mean. Statistical significance is indicated by an asterisk ($p < 0.05$).

Figure 6: Serum Electrolytes in RLY5016-treated Pigs with Normal Renal Function



Symbols represent the daily value from Day 10 for each individual animal. The horizontal bar represents the group mean.

Table 5: Fecal and Urine Electrolytes after 8 Days of Treatment with RLY5016

Treatment Group	Potassium (mEq/day) (Mean ± SD)		Sodium (mEq/day) (Mean ± SD)		Magnesium (mEq/day) (Mean ± SD)		Calcium (mEq/day) (Mean ± SD)	
Fecal Electrolytes								
Days	**1**	**3 – 8**	**1**	**3 – 8**	**1**	**3 – 8**	**1**	**3 – 8**
Untreated (N = 7)	31.2 ± 5.5	37.4 ± 7.8	17.1 ± 4.8	21.3 ± 4.5	60.1 ± 9.3	78.2 ± 11.2	79.9 ± 13.8	95.5 ± 13.6
RLY5016 (N = 7)	27.0 ± 7.2	45.3 ± 5.3	16.6 ± 9.4	42.2 ± 5.6	48.5 ± 14.8	75.4 ± 7.8	58.6 ± 11.8	150.3 ± 14.0
p-value	ns	< 0.05	ns	< 0.0001	ns	ns	< 0.05	< 0.0001
Urine Electrolytes								
Days	**1 – 8**		**1 – 8**		**1 – 8**		**1 – 8**	
Untreated (N = 7)	88.9 ± 15.5		144.8 ± 19.2		18.7 ± 4.1		1.9 ± 0.8	
RLY5016 (N = 7)	71.8 ± 9.7		115.2 ± 16.7		17.8 ± 6.0		26.8 ± 10.1	
p-value	< 0.05		< 0.05		ns		< 0.0001	

ns = not statistically significant
p-values calculated using a 2-tailed t-test versus the untreated group

Table 6: Serum Electrolytes after 8 Days of Treatment with RLY5016 (Day 10)

Treatment Group	Potassium (mmol/L) (Mean ± SD)	Sodium (mmol/L) (Mean ± SD)	Magnesium (mmol/L) (Mean ± SD)	Calcium (mmol/L) (Mean ± SD)
Untreated (N = 6)	4.9 ± 0.7	147.2 ± 1.3	0.8 ± 0.1	2.8 ± 0.1
RLY5016 (N = 4)	4.6 ± 0.6	146.5 ± 1.0	0.8 ± 0.1	2.9 ± 0.2
p-value	ns	ns	ns	ns

ns = not statistically significant
p-values calculated using a 2-tailed t-test versus the untreated group

4.1.2.3 Pharmacologic Effects of RLY5016 in a Chronic Renal Failure Rat Model (TR 300-07-027)

A variety of animal models have been used to mimic the pathogenesis of chronic renal failure; however, few models are available for chronic renal failure-associated hyperkalemia. Therefore, Relypsa developed a new rat hyperkalemia model, designated NADR-TQ, in which chronic renal failure was induced via subtotal (5/6 th) nephrectomy followed by a single intravenous injection of Adriamycin (3.5 mg/kg) at 2 weeks post-nephrectomy. A hyperkalemic state was induced by treatment with trimethoprim (0.3% w/w in the chow) and quinapril (30 mg/L in the drinking water) immediately after Adriamycin injection. Rats with normal renal function have a serum potassium level of 6.00 ± 0.65 mmol/L, while nephrectomized rats injected with Adriamycin that were not exposed to quinapril and trimethoprim showed a slight but significant elevation of serum potassium at 2 weeks post-Adriamycin injection, 6.58 ± 0.65 mmol/L ($p < 0.01$ relative to normal controls). The combination of trimethoprim and quinapril as supplements to the diet of the nephrectomized rats injected with Adriamycin further exacerbated the hyperkalemia, resulting in a persistent and progressive hyperkalemia. The serum potassium level in the NADR-TQ rats, at Days 7 and 14, was 7.08 ± 0.89 and 7.26 ± 0.68 mmol/L, respectively ($p < 0.001$ relative to normal controls).

Male Sprague-Dawley rats in which chronic renal failure had been induced by subtotal nephrectomy (10 per group) were randomly assigned to receive RLY5016 (4% w/w [2.6 g/kg/day] in chow) or chow alone. Serum samples were collected from all rats 5 days prior to Adriamycin injection and on Days 7 and 14 post-Adriamycin injection. Twenty-four (24)-hour urine and fecal samples were collected, and body weight and food and water consumption were assessed at Days -1, 7 and 14 post-Adriamycin injection.

At Day 7, RLY5016-treated NADR-TQ rats demonstrated significantly lower serum potassium levels than untreated NADR-TQ rats (6.4 ± 0.8 versus 7.6 ± 0.7 mmol/L, $p < 0.001$). Serum potassium levels were within the normal range (6.00 ± 0.65 mmol/L) in the treated animals, while untreated NADR-TQ rats became hyperkalemic (Figure 7). At Day 14, the serum potassium levels in the RLY5016-treated group were also lower than untreated NADR-TQ rats, but the difference was not statistically significant (data not shown).

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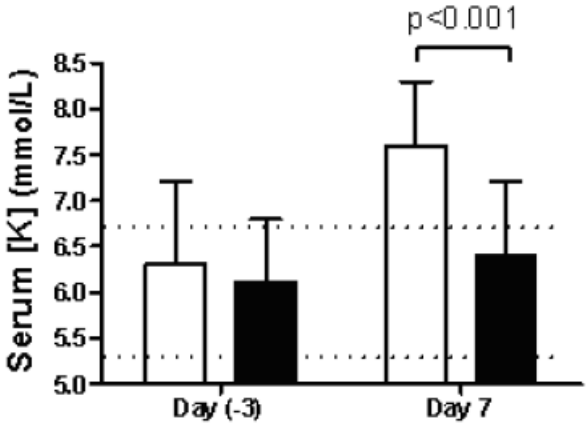
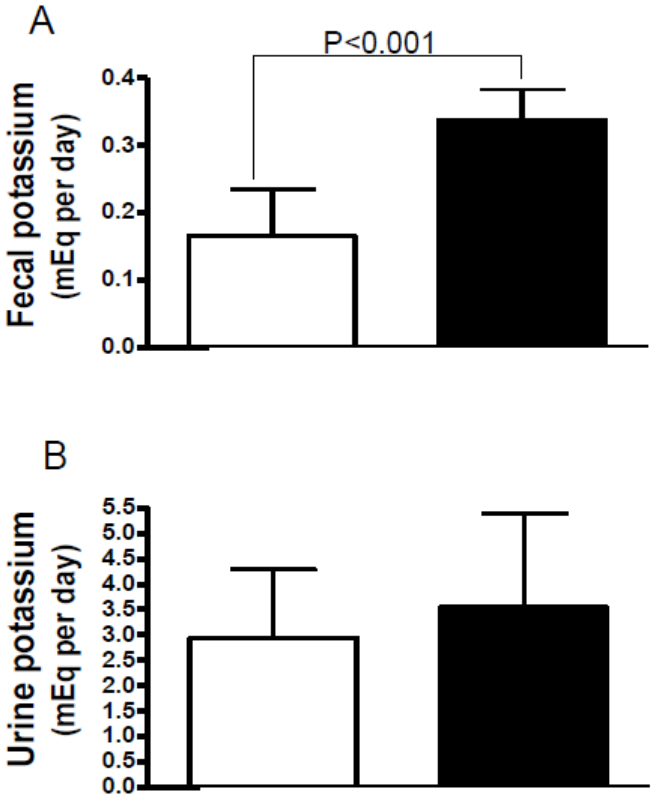
Figure 7: RLY5016 Attenuates Hyperkalemia in NADR-TQ Rats



Open bars = NADR-TQ rats; Closed bars = NADR-TQ rats treated with 4% w/w RLY5016 in chow
Mean ± SD; N = 10 per group
Horizontal dotted lines mark the normal serum potassium range for male Sprague-Dawley rats of the same age.

After 14 days of treatment, NADR-TQ rats receiving RLY5016 had significantly ($p < 0.001$) greater fecal potassium excretion than untreated NADR-TQ rats (0.33 ± 0.06 mEq potassium/day vs. 0.15 ± 0.08 mEq potassium/day; Figure 8A). For purposes of comparison, rats with normal renal function excrete approximately 0.04 mEq potassium/day, whereas nephrectomized animals injected with Adriamycin without trimethoprim and quinapril supplements excrete approximately 0.12 mEq potassium/day (data not shown). In contrast, there was no effect of RLY5016 treatment on urinary potassium excretion in NADR-TQ rats (Figure 8B). Thus, the increased fecal potassium excretion observed in RLY5016-treated animals was not a compensation for decreased urinary secretion, the net effect being reduction in total body potassium and normalization of serum potassium levels. These results suggest that RLY5016 acts as a potassium “sink” to augment the adaptive potassium secretion in the colon noted during chronic renal failure.

Figure 8: RLY5016 Effects on Fecal and Urinary Potassium Excretion in NADR-TQ Rats



Open bars = NADR-TQ rats; Closed bars = NADR-TQ rats treated with 4% w/w RLY5016 in chow
Mean ± SD; N = 10 per group
Data are from Day 14

There were no significant effects of RLY5016 on food or water consumption or on body weight, urine volume or fecal volume in this study.

4.2 Secondary Pharmacology

No secondary pharmacodynamic studies were conducted.

4.3 Safety Pharmacology

Five safety pharmacology studies compliant with Good Laboratory Practices (GLP) were conducted with oral administration of RLY5016 at doses up to 6 g/kg in rats or 3.5 g/kg in dogs. All studies were reviewed under IND 75615.

A study of the central nervous system effects of RLY5016, including observations of general behavior, autonomic and motor effects as assessed by the Irwin method (Irwin, 1968), was performed in male Wistar rats. Studies of the potential effects of RLY5016 on pulmonary function and GI motility were also conducted in

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male Wistar rats, and the effects of the drug on cardiovascular parameters were explored in telemetry-implanted beagle dogs.

Results of these studies indicated no adverse effects in any of the safety parameters tested. However, in the rat, RLY5016 at a dose of 3 g/kg produced a slight, but statistically significant decrease in GI transit, and doses of 3 and 6 g/kg produced a statistically significant inhibitory effect on stomach emptying. These doses are approximately 3- and 6-fold higher (on weight to weight basis) than the proposed maximum daily clinical dose of RLY5016 for Oral Suspension ((b) (4)). Given the very large doses of RLY5016 administered in this study, residual polymer in the stomach could be contributing to the significant increase in the stomach plus content weights observed.

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5 Pharmacokinetics/ADME/Toxicokinetics

Brief Summary

RLY5016 for Oral Suspension is a powder dosage form that has been developed by Relypsa for the treatment of hyperkalemia. RLY5016S is the drug substance used in the manufacture of the drug product, which comprises (b) (4)% RLY5016S and (b) (4)% xanthan gum. RLY5016S is a free-flowing powder of small (on average approximately (b) (4) spherical beads. The drug substance consists of the polymer anion (the active moiety) and a calcium-(b) (4). The active moiety is a nonabsorbed cation-exchange polymer that binds potassium in the lumen of the colon and increases fecal potassium excretion, leading to removal of potassium from the body and lowering of serum potassium levels.

In addition to RLY5016S, pharmacokinetic (PK) studies conducted in support of this application used (b) (4) other forms of the drug substance. The test articles RLY5016 and RLY5016 (b) (4) refer to the polymer anion (b) (4), respectively. Each of these test articles contains the polymer anion, which is the active moiety of RLY5016S. The nomenclature used for expressing the dose of drug product administered changed during the development program. Some of the nonclinical studies described in this section expressed dose in terms of RLY5016 (b) (4)

5.1 PK/ADME

5.1.1 Brief summary

5.1.1.1 PK /ADME

Two studies have been conducted to assess the extent of RLY5016 bioavailability, one in rats (TR 350-07-007) and one in dogs (TR 350-07-008). RLY5016 was radiolabelled with (b) (4) crosslinking agent that is a component of the finished (b) (4) beads. The labeled polymer was administered to the test animals and plasma, fecal and urinary monitoring was conducted to assess possible absorption and distribution of the drug. Analysis showed that 0.004% and 0.002% of the administered radiolabel was present in plasma in the rat and dog, respectively. Quantitative whole body autoradiography in rats did not detect any radiolabel outside the gastrointestinal (GI) tract. Recovery of the radiolabel in feces and urine was 84.1% and 0.15%, respectively, in rats and 99.9% and < 0.1%, respectively, in dogs. The lower fecal recovery observed in rats was attributed to difficulty collecting and homogenizing rat feces. The amount of radiolabel recovered in the urine of both species was consistent with the level of unincorporated (b) (4) extracted (b) (4)

the total specific activity). Furthermore, the majority of the unincorporated radiolabel (97.87%) was found in a single peak that co-eluted with the (b) (4) (b) (4) used in the synthesis of the RLY5016 bead, providing evidence that the radioactivity observed in the urine of rats and dogs was due to unincorporated (b) (4) rather than absorbed RLY5016 beads (Report No. 670402, titled "Determination of (b) (4) (b) (4) and its Related Impurities in ILY105 Beads"). Therefore, studies in both the rat and the dog demonstrated the nonabsorbed nature of the polymer and its lack of systemic bioavailability.

RLY5016 is stable and does not degrade during passage through the GI tract. As described in RLY-TR-0054, titled "Analysis of Cation Content in RLY5016 Isolated from Human Feces", the physical stability of the RLY5016 beads during transit through the GI tract was demonstrated by recovering the product from fecal samples collected from human subjects in Study RLY5016-101, titled "A Double-Blind, Randomized, Placebo-Controlled, Parallel-Group Single and Multiple Dose Escalation Study to Evaluate the Safety and Tolerability of RLY5016 in Healthy Volunteers". The beads recovered from feces remain as intact, (b) (4) spheres. The metabolic stability of the beads was demonstrated by analysis of cation content of the recovered RLY5016 beads: at all doses, the sum of the major physiologic cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) bound to the recovered polymer beads equaled the amount of (b) (4) contained on beads that had not passed through the GI tract. Thus, RLY5016 is not metabolized or changed by GI passage, as loss of cation binding moieties due to such metabolism would be reflected in altered cation content.

5.1.1.2 Drug-drug Interactions

Since RLY5016S is not systemically absorbed, drug-drug interactions (DDIs), if they occur, would occur through binding of the polymer to another orally administered drug in the GI tract leading to a change in the absorption of the interacting drug. As agreed with the Agency at the pre-NDA Meeting (Minutes of 04 Mar 2014 pre-NDA Meeting), a biologically-relevant *in vitro* test system was used to evaluate potential interactions between RLY5016S and a set of 28 orally administered compounds commonly used in the target patient population for RLY5016 for Oral Suspension (Study RLY-TR-0130). The set of compounds tested included prototypical drugs from many drug classes, as well as vitamins and a hormone. The set of Test Drugs used for assessing DDIs with RLY5016S was agreed with the Agency at the pre-NDA meeting held 04 March 2014 (Minutes of 04 Mar 2014 pre-NDA Meeting). In addition, a quantitative structure-property relationship (QSPR) based model was developed that reliably predicts the binding of drugs to RLY5016S.

The conclusions from these two studies are that binding of other orally administered compounds to RLY5016S can be predicted based on a small number of readily computed parameters from the molecular structure of the drug alone. These are the:

- computed surface area of acceptor atoms (i.e., summation of the total surface area of atoms identified to be hydrogen bond acceptors),
- ionization potential (the energy of the highest occupied molecular orbital), and electron affinity (the energy of the lowest unoccupied molecular orbital), and
- lipophilicity.

Given the nature of RLY5016S (i.e., a cation binder), the parameters found to be significant in the models are physically reasonable because they describe both the polarity and lipophilicity of the molecules, both of which have long been implicated in small molecule interactions with various proteins and polymers.

The results of the *in vitro* DDI study and the QSPR modeling are adequate to propose specific dosing recommendations in the label to avoid untoward drug interactions with RLY5016 for Oral Suspension.

5.1.1.3 Fluoride Absorption

The anion of RLY5016S is a crosslinked polymer of 2-fluoro(b) (4). Fluoride degradation of RLY5016 for Oral Suspension has been demonstrated in stability studies to occur at a rate of approximately (b) (4) ppm per month under refrigerated conditions. (b) (4) Any fluorine that dissociates from the polymer will likely form the insoluble calcium fluoride (CaF_2) salt. Calcium fluoride is much less bioavailable than sodium fluoride (NaF) because it is much less water soluble ((b) (4), respectively). In human and animal studies, the bioavailability of fluoride from CaF_2 is lower than that of NaF (Trautner, 1987; Jowsey, 1978; Whitford, 1994).

Studies using a rat model were conducted to test the hypothesis that minimal fluoride absorption would result from the oral administration of RLY5016 (TR 300-07-028). Rats were orally administered RLY5016 (b) (4) and RLY5016 (b) (4) such that they received a comparable level of fluoride impurity from each compound, and the PK profiles of fluoride were subsequently assessed in plasma. The results demonstrated that RLY5016 gave a (b) (4) lower maximum fluoride concentration (C_{max}) and a (b) (4) lower fluoride area under the curve (AUC) compared to RLY5016 (b) (4) when analyzing equivalent doses. These data demonstrate that oral administration of the polymer anion complexed with calcium results in systemic

absorption of fluoride that is substantially lower than that observed with the sodium salt form of the drug. This is consistent with previous studies using fluoride co-administered with calcium (b) (4)

5.1.2 Methods of Analysis

5.1.2.1 Radiochemical Procedures
Biological samples obtained from rat and dog studies conducted with [^{14}C]-RLY5016 were used to determine the extent of absorption, distribution and excretion of the radiolabel (TR 350-07-007 and TR 350-07-008). Samples were assessed for ^{14}C content by combustion analysis using a Model (b) (4) Sample Oxidizer (b) (4) and the resulting $^{14}CO_2$ was trapped in a mixture of (b) (4) scintillation cocktail was used for samples analyzed directly. All samples were analyzed for radioactivity in a Model (b) (4) liquid scintillation counter ((b) (4)) for at least 5 minutes or 100,000 counts. Each sample was homogenized before radioanalysis (unless the entire sample was used for analysis). All samples were tested in duplicate if sample size allowed. If results from sample duplicates (calculated as ^{14}C dpm/g sample) differed by more than 10% from the mean value, the sample was re-homogenized and reanalyzed (if the sample size permitted). Scintillation counting data (cpm) were automatically corrected for counting efficiency using the external standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards.

Whole body autoradiography for the rat ADME study was conducted as follows. Prior to section collection, quality control standards fortified with ^{14}C radioactivity were placed into the frozen block containing the rat carcass and were used for monitoring the uniformity of section thickness. Appropriate sections were collected on adhesive tape at 40 micrometer thickness, using a Leica (b) (4) cryomicrotome in accordance with (b) (4) Standard Operating Procedures. Sections were collected at five levels of interest in the sagittal plane. All major tissues, organs and biological fluids were represented. Collected sections were dried at approximately -20°C. A section set from each rat was prepared by mounting a representative section from each level of interest. Mounted sections were tightly wrapped with Mylar film and exposed on phosphorimaging screens along with plastic-embedded autoradiographic standards purchased from (b) (4) for subsequent calibration of the image analysis software. Screens were exposed for 4 days and then scanned using an (b) (4). The autoradiographic standard image data were sampled using (b) (4) AIS software to create a calibrated standard curve. Using this standard curve, a lower limit of detection based on the lowest readable concentration was calculated (reported as the lower limit of quantitation based on the mean). Specified tissues, organs and fluids were analyzed. Using AIS software, tissue concentrations were interpolated from each standard curve as nanocuries/g and then converted to microgram equivalents/g

on the basis of the test article specific activity. Tissue concentration data were summarized in tabular format. Autoradiographs were annotated and printed on a FUJIX Pictography 3000 digital image printer.

5.1.2.2 High-Performance Liquid Chromatographic Procedures
For 24 of the Test Drugs (allopurinol, amlodipine, amoxicillin, apixaban, aspirin, cephalexin, cinacalcet, ciprofloxacin, clopidogrel, furosemide, glipizide, lisinopril, metformin, metoprolol, phenytoin, quinidine, riboflavin, rivaroxaban, spironolactone, thiamin, trimethoprim, valsartan, verapamil and warfarin), a reversed phase high performance liquid chromatography (HPLC) method (Method 13RA01) was used for analysis in the three test matrices. The method utilized two different HPLC gradients, depending on the chromatographic properties of the Test Drug, and UV detection at 214 or 270 nm. Quantitation was performed for each Test Drug using a single-point calibration standard. A (b) (4) HPLC system equipped with an autosampler and dual-wavelength detector was used. The columns used were: 50 x 2.0 mm Luna C18(2), 3 µm (b) (4) and 50 x 2.0 mm Proto 200 C18, 5 µm (b) (4) . Qualification of the assay was performed for each Test Drug for the following parameters: specificity, linearity, accuracy and precision, and stability for each analyte; results are presented in RLY-TR-0130, Attachment 2 (RA-001-RPT), Attachment 3 (RA-024-RPT) and Attachment 4 (RA-028-RPT).

Atorvastatin and digoxin were measured in the three test matrices using liquid chromatography-mass spectrometry (LCMSMS) methods in positive electrospray ionization mode (ESI+; Method RA-012-TMA for atorvastatin and Method RA-005-TMA for digoxin). The methods used an (b) (4) tandem quadrupole mass spectrometer equipped with a TurbolonSpray probe for ESI+ ionization and a (b) (4) HPLC equipped with an SIL-HT autosampler. The columns were: Proto C18 (b) (4), 20 x 2.1 mm, 5 µm (b) (4) and Proto C18, 20 x 2.1 mm, 5 µm guard cartridge (b) (4)). The atorvastatin method incorporated the processing of 5-µL aliquots of sample (b) (4) Quantitation was performed using a single point calibration standard in both the atorvastatin and digoxin methods. Qualification of the two LCMSMS assays was performed for the following parameters: specificity, linearity, accuracy and precision, and stability; results are presented in RLY-TR- 0130, Attachment 5 (RA-009-RPT; digoxin) and Attachment 6 (RA-017-RPT; atorvastatin).

[redacted] was measured in the three test matrices using ion chromatography (IC) with isocratic conditions (Method RLY-AM-0029). The method used a [redacted] autosampler, conductivity detector CD25 and DS3 flow cell. The column was a [redacted] 250 x 4 mm analytical column with a [redacted] 50 x 4 mm guard column. [redacted] samples were not diluted prior to analysis. Quantitation was performed using a single-point calibration standard. Qualification of the IC method was performed for the following parameters: specificity, linearity, accuracy and precision, and stability; results are presented in RLY-TR-0130, Attachment 8 (RLY-TR-0146).

5.1.2.3 Ion-specific Electrode Procedure
Plasma fluoride was analyzed using a fluoride ion-specific electrode following hexamethyldisiloxane (HMDS)-facilitated diffusion (Taves, 1968; Whitford, 1996). Briefly, HMDS-facilitated diffusion transfers [redacted] A fluoride ion-specific electrode is used to measure the fluoride content in the adjusted sample.

5.1.3 Absorption
RLY5016S was designed to be a nonabsorbed polymeric drug that binds potassium in the lumen of the colon. The polymer is synthesized using a [redacted] RLY5016S is manufactured as spherical beads that are approximately [redacted] in diameter. This particle size is large enough to prevent absorption via transcellular or paracellular routes through intestinal epithelial cells (the cells themselves are approximately 50 – 100 micrometers in diameter), but small enough to prevent the grittiness often associated with polymer materials made by [redacted] There is an extensive body of literature examining the absorption kinetics of various sized [redacted] beads that demonstrates that particles greater than approximately one micrometer in size are not detected in the blood and do not accumulate in the liver, spleen, bone marrow or kidney (Jani, 1989; Jani, 1990; Jung, 2000). As shown in Figure 1, the particle size distribution of RLY5016 manufactured for the absorption studies ranged from approximately [redacted]. Specifically, the bead sizes ranged from [redacted] which is also within current specification limits for RLY5016S. Thus, the size distribution of RLY5016 beads is well in excess of the maximum size for intestinal absorption of a [redacted] bead.

Figure 1: Particle Size Distribution of RLY5016 Beads

Given the size of the RLY5016 polymer, systemic absorption of the drug was not expected. However, two ADME studies were conducted to verify this expectation, one in rats (TR 350-07-007) and one in dogs (TR 350-07-008). RLY5016 was radiolabelled with (b) (4) crosslinking agent that is a component of the finished (b) (4) beads (see Section 3.2.S.2.2 [RLY5016S, (b) (4)]). The specific activity of the radiolabeled beads (Lot No. EPPS-05-032-31-24, Certificate of Analysis can be found in Report No. 675500), determined by combustion analysis, was (b) (4). Plasma, fecal and urinary monitoring was conducted to assess possible absorption and distribution of the drug.

5.1.3.1 Single Dose Rat ADME Study (TR 350-07-007)
Nine male Sprague Dawley rats were administered a single dose of ^{14}C-RLY5016 at 313 mg/kg (107 µCi/kg) via oral gavage. Blood was collected from three rats per collection time point, at 0.25, 0.5, 1, 2, 4, 8, 24, 48 and 72 hours postdose. Whole blood and plasma were analyzed for radioactivity by liquid scintillation counting (LSC). In whole blood, all time points yielded levels of radioactivity that were below the limit of quantitation (BLQ; set at twice the background level of radiation in this study), with the exception of one rat at each of three time points. No individual rat showed more than one whole blood measurement that was above the quantitation limit. Very low levels of radioactivity were detected in plasma samples (Table 1) beginning at 1 hour post-dose and returning to BLQ levels after 24 hours post-dose. The highest average plasma level was observed 8 hours after dosing. Assuming an average plasma volume of 4.16 mL/100 g (Lee, 1985) and an average rat body weight of 205 g (TR 350-07-007, Table 1), the highest average total plasma level of

radiolabel observed in the study was 0.004% of the dose administered (2.78 micrograms compared to a 64.2 mg dose). The chromatographic data provide evidence that the small amount of radiolabel detected in plasma is from unincorporated labeled crosslinker ([redacted] (b) (4)) and not from the labeled polymer itself. Thus, physiologically relevant PK parameters could not be derived for RLY5016 from the data obtained in this study.

Table 1: Radioactivity in Plasma after Single Oral Dose ^{14}C-RLY5016 Administration in Rats

Time Point (hours)	Microgram Equivalents ^{14}C-RLY5016/g Plasma			
	Individual			Mean (SD)
	1	2	3	
0.25[a]	BLQ	BLQ	BLQ	-
0.5[b]	BLQ	BLQ	BLQ	-
1[c]	0.209	0.170	0.204	0.194 (0.021)
2[a]	0.282	0.139	0.376	0.266 (0.119)
4[b]	0.309	0.139	0.171	0.207 (0.090)
8[c]	0.384	0.346	0.248	0.326 (0.070)
24[a]	0.0969	BLQ	0.161	0.0861 (0.0812)
48[b]	BLQ	BLQ	BLQ	-
72[c]	BLQ	BLQ	BLQ	-

BLQ = below limit of quantitation (set to < 2x background and replaced with zero for calculation of means); SD = standard deviation; "-" = mean not calculated

[a] Blood collected from animal numbers B00621 – B00623

[b] Blood collected from animal numbers B00624 – B00626

[c] Blood collected from animal numbers B00627 – B00629

5.1.3.2 Single Dose Dog ADME Study (TR 350-07-008)
Three male and three female beagle dogs were administered a single oral dose of ^{14}C-RLY5016 (350 mg/kg; 105 microCi/kg). Blood samples were collected predose and at 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120 and 168 hours postdose. Whole blood and plasma were analyzed for radioactivity by LSC.

Oral absorption of ^{14}C-RLY5016 following the single dose administration was minimal. In whole blood samples, radioactivity was detected sporadically, with levels above the quantitation limit observed in all six dogs at only the 4-hour postdose time point. Very low levels of radioactivity were detected in plasma samples (Table 2). The mean plasma C_{max} values were 0.339 and 0.406 microgram equivalents ^{14}C-RLY5016/g for males and females, respectively, at 8 and 4 hours postdose (T_{max}). The maximum amount of radioactivity in plasma was estimated to be approximately 0.002% of the total administered dose. As was discussed for the rat study, chromatographic data provide evidence that the small amount of radiolabel detected in dog plasma is from unincorporated

labeled crosslinker ([redacted](b) (4)) and not from the labeled polymer itself. Despite the fact that the plasma concentration data observed in this study are likely not a measure of absorption of the RLY5016 beads, PK parameters were calculated in the dog study and are presented here in Table 3.

Table 2: Radioactivity in Plasma after Single Oral Dose ^{14}C-RLY5016 Administration in Dogs

Time Point (hour)	Microgram Equivalents ^{14}C-RLY5016/g Plasma							
	Males				Females			
	1	2	3	Mean (SD)	1	2	3	Mean (SD)
0.25	BLQ	BLQ	BLQ	-	BLQ	BLQ	BLQ	-
0.5	BLQ	BLQ	BLQ	-	BLQ	BLQ	BLQ	-
1	BLQ	BLQ	BLQ	-	0.0594	BLQ	BLQ	0.0198 (0.0343)
2	0.176	0.103	BLQ	0.0928 (0.0882)	0.263	0.124	0.0667	0.151 (0.101)
4	0.256	0.394	0.240	0.297 (0.0850)	0.598	0.324	0.296	0.406 (0.167)
8	0.185	0.509	0.323	0.339 (0.163)	0.378	0.203	0.233	0.271 (0.0930)
24	0.227	0.312	0.304	0.281 (0.0470)	0.207	0.172	0.163	0.180 (0.0230)
48	0.191	0.227	0.312	0.244 (0.0620)	0.151	0.104	0.121	0.125 (0.0240)
72	0.125	0.188	0.249	0.187 (0.0620)	0.123	0.0885	0.0840	0.0986 (0.0216)
96	0.122	0.159	0.201	0.160 (0.0390)	0.109	0.0762	0.0688	0.0848 (0.0217)
120	0.102	0.130	0.156	0.129 (0.0270)	0.0734	BLQ	0.0702	0.0479 (0.0415)
168	0.0823	0.105	0.132	0.106 (0.0250)	0.0638	BLQ	BLQ	0.0213 (0.0368)

BLQ = below limit of quantitation (replaced with zero for calculation of means); SD = standard deviation; "-" = mean not calculated

Limit of quantitation in plasma is 0.0617 microgram equivalents ^{14}C-RLY5016/g.

Table 3: Plasma Pharmacokinetic Parameters in Dogs

Parameter	Males Mean (SD)	Females Mean (SD)
T_{max} (hour)	7 (2)	4 (0)
C_{max} (microgram equivalents ^{14}C-RLY5016/g)	0.363 (0.131)	0.406 (0.167)
$t_{1/2}$ (hour)	127 (3)	97.9 (8.27)
$AUC_{(0-t)}$ (microgram equivalents ^{14}C-RLY5016 x hour/g)	31.6 (7.1)	16.6 (6.1)
$AUC_{(0-\infty)}$ (microgram equivalents ^{14}C-RLY5016 x hour/g)	51.0 (11.2)	26.5 (5.1)

$AUC_{(0-t)}$ = area under the plasma concentration time curve from zero to time t; $AUC_{(0-\infty)}$ = area under the plasma concentration time curve from zero to infinity; C_{max} = maximum plasma concentration; SD = standard deviation; $t_{1/2}$ = half-life; T_{max} = time to maximum plasma concentration

5.1.4 Distribution

Tissue distribution of radioactivity associated with single dose administration of ^{14}C-RLY5016 (313 mg/kg; 107 microCi/kg) was measured in rats using whole body autoradiography and analysis of tissue samples (TR 350-07-007). Radioactivity was detected in the following compartments, which correspond to various lumenal segments of the GI tract: cecum contents, large intestinal contents, small intestinal contents and stomach contents. No radioactivity was detected in any other tissues, indicating that the labeled RLY5016 did not cross tissue barriers and stayed within the chyme and fluid moving through the GI tract. After dosing, the earliest radiolabel detection was seen in stomach and small intestinal contents at 0.25 hour postdose and diminished thereafter in these compartments such that no signal was detected after 4 hours postdose. Radioactivity was observed in cecum and large intestinal contents at 1 and 4 hours postdose, slightly overlapping with the upper GI content detection. In all cases, radioactivity was eliminated from the collective GI compartments after 4 hours postdose, and no signal was detected thereafter out to 72 hours postdose.

5.1.5 Metabolism (Interspecies Comparison)

Since RLY5016 is not systemically absorbed, metabolism of the compound does not occur in blood and tissues, and these were not evaluated for metabolites in the rat and dog ADME studies. Instead, the metabolic stability of the RLY5016 polymer was assessed by measuring the cation content (b) (4) recovered from fecal samples obtained from clinical trial subjects (Study RLY5016-101) and comparing this content with the calcium content of the administered RLY5016 dose.

In Study RLY5016-101, 33 healthy subjects on a diet that was controlled for potassium, sodium, calcium and magnesium were administered RLY5016 at doses of 1, 5, 10 or 20 g three times daily (TID) and total urine and feces were collected. This clinical study demonstrated that administration of RLY5016 resulted in a significant dose-dependent increase in fecal potassium excretion (Figure 2), with a corresponding decrease in urinary this potassium excretion (Figure 3).

Figure 2: Fecal Potassium Excretion (All Treated Subjects); Clinical Study RLY5016-101

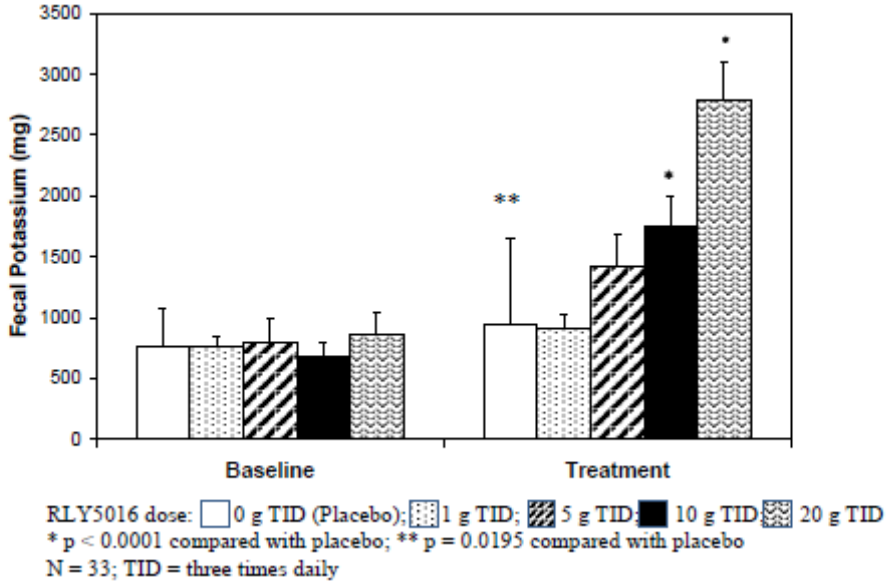
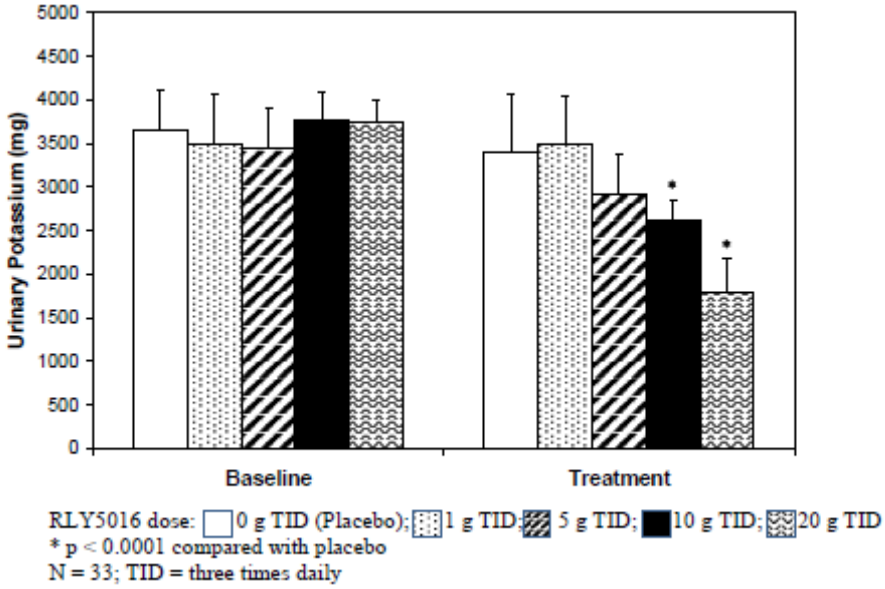


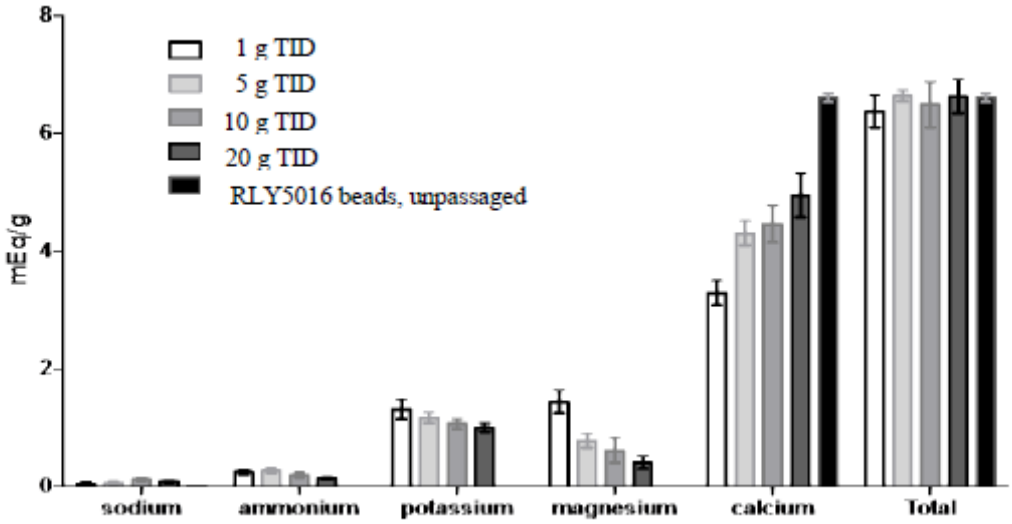
Figure 3: Urinary Potassium Excretion (All Treated Subjects); Clinical Study RLY5016-101



The metabolic stability of the RLY5016 beads can be inferred from assessment of the binding capacity of the polymer before and after transit through the GI tract. The binding capacity of the polymer is dependent upon the carboxylate groups derived from the [redacted](b) (4) that forms the majority of the polymer backbone. Degradation of the polymer, and consequent loss of the carboxylate binding moiety, would result in a loss of binding capacity. In contrast, the beads isolated from subjects administered RLY5016 in Study RLY5016-101 retained their binding capacity after GI transit (Figure 4 and Table 4). The aggregate sum of sodium, ammonium, potassium, magnesium and

calcium bound to the recovered beads was equal to the cation capacity of the unpassaged beads (i.e., prior to administration).

Figure 4: Cation Binding to RLY5016 Beads Isolated from the Feces of Subjects in Clinical Study RLY5016-101



Mean ± standard deviation; TID = three times daily

Table 4: Cation Binding to RLY5016 Beads Isolated from the Feces of Subjects in Clinical Study RLY5016-101

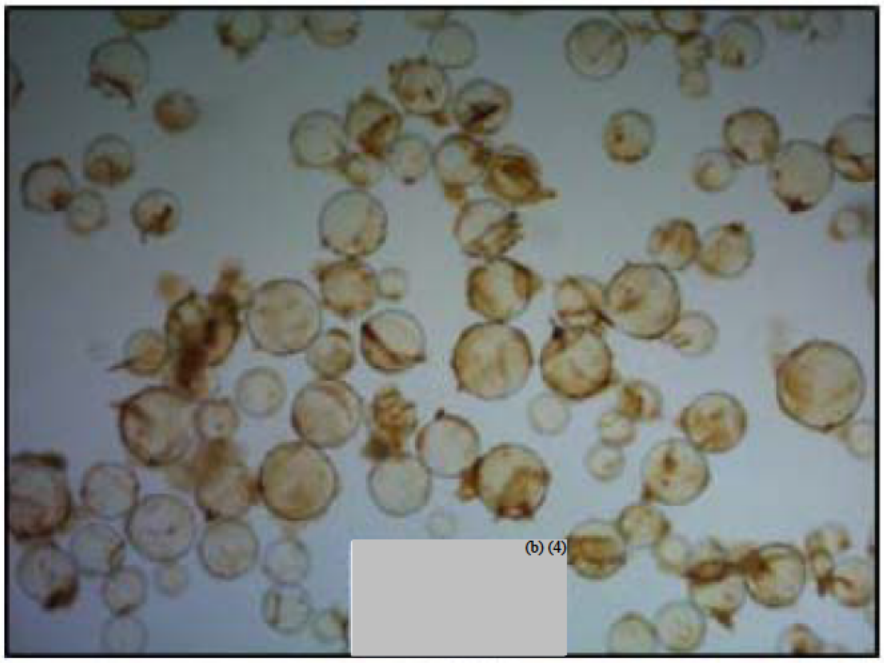
Dose Group	Sodium (mEq/g)	Ammonium (mEq/g)	Potassium (mEq/g)	Magnesium (mEq/g)	Calcium (mEq/g)	Total (mEq/g)
1 g TID	0.05 ± 0.03	0.26 ± 0.09	1.32 ± 0.49	1.44 ± 0.41	3.30 ± 0.20	6.38 ± 0.26
5 g TID	0.07 ± 0.01	0.28 ± 0.11	1.18 ± 0.42	0.79 ±0.18	4.31 ± 0.20	6.64 ± 0.09
10 g TID	0.13 ± 0.02	0.20 ± 0.08	1.08 ± 0.39	0.61 ± 0.22	4.61 ± 0.30	6.64 ± 0.38
20 g TID	0.09 ± 0.02	0.15 ± 0.05	1.01 ± 0.37	0.41 ± 0.10	4.96 ± 0.37	6.63 ± 0.28
RLY5016, unpassaged	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	6.62 ± 0.07	6.62 ± 0.08

Mean ± standard deviation; TID = three times daily

These results demonstrate that RLY5016 serves as a cation-exchange polymer with no net overall cation accumulation or loss during passage through the human GI tract, but with a net binding and uptake of ~1 to 1.3 mEq of potassium per gram of drug administered (Figure 4). Further, the fact that the cation content of the un-passaged beads (6.62 mEq/g polymer) matched that of the recovered beads from the various dose groups (6.38 - 6.64 mEq/g polymer) suggests that there is no net change in the structure of RLY5016 as it passes through the GI tract. Any loss or discontinuity of the [redacted] (b) (4) binding units within the polymer structure would result in a measurable loss of cation binding capacity, which was not observed. Thus, RLY5016 is metabolically stable and is not chemically changed during passage through the GI system.

The physical stability of the RLY5016 beads during transit through the GI tract was demonstrated by recovering the test article from fecal samples collected in Study RLY5016-101 (Figure 5). The beads recovered from feces remained as intact spheres of approximately (b) (4).

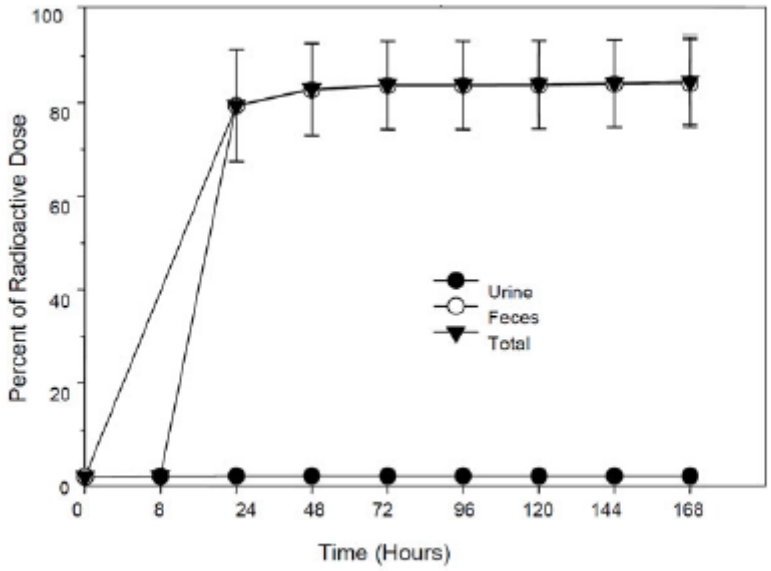
Figure 5: RLY5016 Beads Isolated from Feces of a Representative Subject in Clinical Study RLY5016-101



5.1.6 Excretion

In the rat ADME study (TR-350-07-007), radioactivity was assessed in urine, feces, cage wash, cage wipe and carcasses. Elimination kinetics through urine and feces are shown in Figure 6. Following oral administration of ^{14}C-RLY5016 (313 mg/kg; 107 µCi/kg), the mean total recovery of radioactivity was 84.3%, with 84.1% excreted in feces and 0.15% in urine. Most of the radioactivity was excreted within 48 hours postdose. There was no detectable radioactivity in carcasses by 168 hours postdose. The lower than expected fecal recovery of radioactivity in this study was attributed to difficulty collecting and homogenizing the rat fecal samples; this conclusion is corroborated by the negative (outside the GI tract) results of the whole-body autoradiography described above. There was high variability in the recoveries of radioactivity in feces, suggesting that samples were not homogeneous.

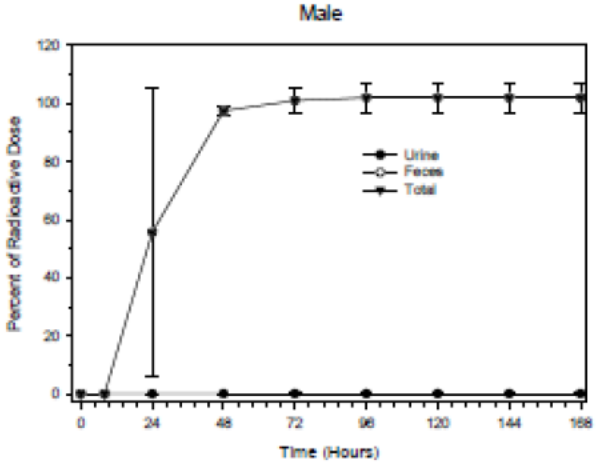
Figure 6: Radioactivity in Urine and Feces after ^{14}C-RLY5016 Administration to Rats



Mean ± standard deviation; N = 3

In the dog ADME study (TR 350-07-008), radioactivity in urine, feces, vomitus/emesis, cage debris, cage wash and cage wipe were measured. Following the oral administration of ^{14}C-RLY5016 (350 mg/kg; 105 µCi/kg), radioactivity was readily eliminated, with most of the radioactive signal excreted from the animals in the urine and feces by 48 hours postdose. Elimination kinetics through urine and feces are shown in Figure 7. Following oral administration of radiolabeled RLY5016, the mean total recovery of radioactivity was 102% in male dogs and 107% in female dogs, with 99.9% of the dose excreted in the feces and < 0.1% excreted in the urine.

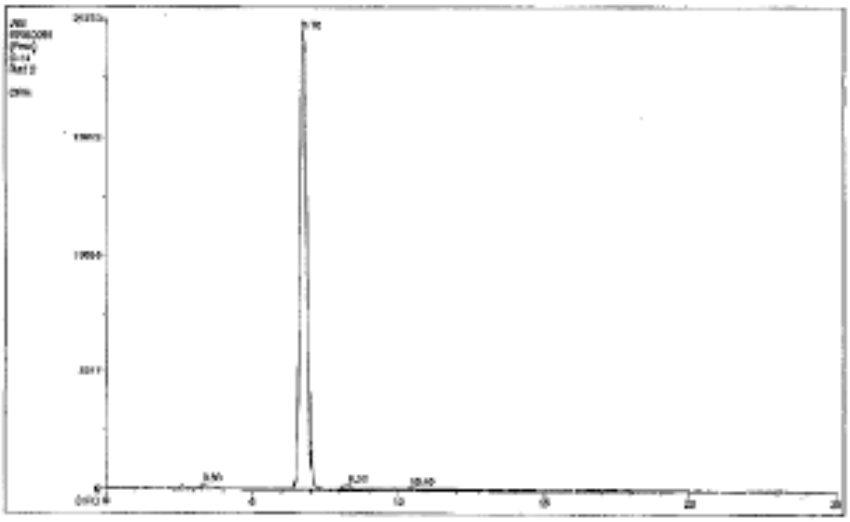
Figure 7: Radioactivity in Urine and Feces after ^{14}C-RLY5016 Administration to Dogs



Mean ± standard deviation; N = 3 males and 3 females

Both the rat and dog ADME studies showed very low levels of radioactivity eliminated in the urine. The following data support the conclusion that the ^{14}C recovered in urine appeared to be unincorporated labeled (b) (4) crosslinker that was systemically absorbed. For these studies, RLY5016 was radiolabeled using a (b) (4) crosslinker incorporated into the RLY5016 beads. (b) (4) consistent with the observed percentage of radioactivity found excreted into the urine in both the rat and the dog. An analysis of impurities in the ^{14}C-labeled RLY5016 beads (Report No. 670402) showed that the majority of the unincorporated radiolabel ((b) (4) %) was found in a single peak (Figure 8). This peak co-eluted with the (b) (4) used in the synthesis of the beads, providing evidence that the radioactivity observed in the urine of rats and dogs was due to unincorporated (b) (4) crosslinker, rather than to absorption of RLY5016 beads.

Figure 8: HPLC Radiochromatogram from an Acetonitrile Extract of [^{14}C]-RLY5016 Beads



5.1.7 Pharmacokinetic Drug Interactions

Since RLY5016S is not systemically absorbed, DDIs, if they occur, would occur through binding of the polymer to another orally administered drug in the GI tract. The most likely effect of binding of RLY5016S to a co-administered drug would be to affect the target compound's absorption through the gastric and/or small intestine compartments. As agreed with the Agency at the pre-NDA Meeting (Minutes of 04 Mar 2014 pre-NDA Meeting), a biologically-relevant *in vitro* test system was used to evaluate potential interactions between RLY5016S and a set of 28 orally administered compounds commonly used in the target patient population for RLY5016 for Oral Suspension. In addition, a QSPR based model was developed that reliably predicts the binding of drugs to RLY5016S.

5.1.7.1 *In vitro* DDI Study

The objective of Study RLY-TR-0130 was to assess the potential for interaction between RLY5016S and a set of 28 orally administered compounds commonly taken by patients who could be prescribed RLY5016 for Oral Suspension. The set of compounds included prototypical drugs from many drug classes, as well as vitamins and a hormone (hereafter all referred to as drugs; see Table 5). Some drugs were included for testing because they have a narrow therapeutic index (e.g., digoxin, levothyroxine, lithium, phenytoin, quinidine and warfarin) or, based on our preliminary understanding of the QSPRs of RLY5016S DDI, they might have been expected to interact with the polymer (i.e., Test Drugs that are basic with pKa(s) > 9.0, have cationic charges, and/or are hydrophilic); Test Drugs without these features were also included in the set of compounds assessed. The drugs evaluated for interaction with RLY5016S included examples from all four Biopharmaceutics Classification System (BCS) classes and a wide range of solubility and lipophilicity. The set of Test Drugs used for assessing DDIs with

RLY5016S was agreed with the Agency at the pre-NDA meeting held 04 March 2014 (Minutes of 04 Mar 2014 pre-NDA Meeting).

Table 5: Drugs Tested *in vitro* for Interaction with RLY5016S

Test Drug	Drug Class	BCS Class	pKa	LogP
Allopurinol	Xanthine oxidase inhibitor	III	9.18A, 12.12A	-0.3 (N)
Amlodipine besylate	Calcium channel blocker	I	9.21B	3.39 (N), 1.44 (C)
Amoxicillin	Penicillin antibiotic	III	2.60A, 7.35B, 9.59A	-0.02 (N), 2.19 (C)
Apixaban	Factor Xa inhibitor	III	NA	< 1.40 (N)
Aspirin	Salicylate	III	3.50A	0.90 (N)
Atorvastatin calcium	Statin	II	4.47A	4.01 (N), 1.04 (A)
Cephalexin	Cephalosporin antibiotic	III	2.56A, 7.10B	-1.05 (N)
Cinacalcet HCl	Calcimimetic	IV	8.85B	5.58 (N), 2.41 (C)
Ciprofloxacin HCl	Quinolone antibiotic	IV	6.35A, 8.33B	-0.27 (N)
Clopidogrel bisulfate	Antiplatelet	II	4.66B	4.06 (N), 0.95 (C)
Digoxin	Cardiac glycoside	II	NA	1.64 (N)
Furosemide	Diuretic	IV	3.62A, 10.16A	2.20 (N), -0.82 (A)
Glipizide	Sulfonylurea	II	5.06A	2.91 (N), -0.45 (A)
Levothyroxine	Synthetic oral hormone	II	2.00A, 6.65A, 8.73B	3.44 (N)
Lisinopril	Angiotensin converting enzyme inhibitor	III	1.63A, 3.13A, 7.13B, 10.75B	-0.51 (N)
Lithium carbonate	Bipolar Treatment	I	NA	NA
Metformin HCl	Biguanide	III	2.94B, 13.7B	< -1.50 (N)
Metoprolol tartrate	Beta blocker	I	9.61B	1.91 (N)
Phenytoin sodium	Anticonvulsant/ Antiarrhythmic	II	8.18A	2.43 (N), -0.09 (A)
Quinidine gluconate	Antiarrhythmic/ Antimalarial	I	4.39B, 9.06B	3.75 (N), 0.85 (C)
Riboflavin	B Vitamin	I	9.87A	< -1.50 (C)
Rivaroxaban	Factor Xa inhibitor	II	NA	1.43 (N)
Spironolactone	Aldosterone receptor antagonist	II	NA	2.53 (N)
Thiamin HCl	B Vitamin	III	4.88B	< -1.50 (C)
Trimethoprim	DHFR inhibitor antibiotic	II	7.14B	0.78 (N)
Valsartan	Angiotensin II receptor blocker	II	3.73A, 4.40A	3.98 (N), 1.48 (A)
Verapamil HCl	Calcium channel blocker	I	8.95B	4.20 (N), 0.52 (C)
Warfarin	Anticoagulant (Vitamin K antagonist)	II	4.94A	3.25 (N), -0.77 (A)

A = acid; B = base; BCS = Biopharmaceutics Classification System; DHFR = dihydrofolate reductase; HCl = hydrochloride; NA = not applicable; (A) = anionic; (C) = cationic; (N) = neutral

Note: pKa and logP values are from Report No. SIRIUSRELYPSA01, titled "pKa and logP study for Relypsa".

It was assumed that the likelihood of identifying a drug interaction would be greatest at the maximum dose of RLY5016S and the minimum dose of the Test Drug. Therefore, the study used the maximum single dose of RLY5016S used in human clinical trials (equivalent to 25.2 g polymer anion) and the lowest single human dose of Test Drug, where feasible. In cases where the Test Drug was not soluble in the test matrices, the concentration of the Test Drug was reduced from the lowest single human dose until solubility was achieved. While the concentration of an orally administered drug varies dynamically on a temporal, inter- and intra-individual basis, based on publications (Metcalf, 1987; Read, 1980; Thelen, 2011), a volume of 1 liter was considered an appropriate representative volume for dispersion of an orally administered drug in the upper GI tract. Therefore, the study was performed at concentrations of 25.2 g/L polymer anion and the lowest single human dose of Test Drug described in US prescribing information, per liter. Smaller or larger volumes would not change the relative concentrations of RLY5016S and the co-administered drugs.

Interactions between RLY5016S and the drugs listed in Table 5 (Test Drugs) were assessed by measuring the free Test Drug concentration after incubation in the presence or absence of RLY5016S.

Three test matrices were used: SGF + 0.05% Tween-20 (pH 1.2), Acetate Buffer + 0.05% Tween-20 (pH 4.5) and SIF + 0.05% Tween-20 (pH 6.8). These matrices have previously been used for *in vitro* DDI evaluations of another polymer therapeutic, Welchol™ (colesevelam HCl) (Walker, 2009); all three matrices are representative of pH conditions in the gut. When RLY5016S is added to the matrix at the test concentration of 25.2 mg/mL polymer anion, the pH of the matrix changes; this effect of RLY5016S varies depending upon the concentration of RLY5016S and the concentration of the acidic, basic and other neutral species in the matrix (Burke, 2003; Vink, 1986). In the presence of 25.2 mg/mL polymer anion, the pHs of the three matrices are 3.0, 4.6 and 5.9 for SGF + 0.05% Tween-20, Acetate Buffer + 0.05% Tween-20 and SIF + 0.05% Tween-20, respectively. Since RLY5016 for Oral Suspension is intended to be taken with food, the binding matrices used in this study provide a useful representation of pH conditions in the fed stomach and upper small intestine, which are the conditions and location most important for evaluation of RLY5016S DDIs (Clarysse, 2008; Dressman, 1990; Kalantzi, 2006; Jantratid, 2008).

Based on results from pilot studies, Tween-20 was selected as the surfactant to be included in the test matrices to aid in the solubility of the Test Drugs. The concentration of Tween-20 used was 0.05%, which is close to the critical micelle concentration of 0.01%; this concentration was chosen because it was unlikely to interfere with the analysis of Test Drug concentrations. The use of 0.05% Tween-20 in the test matrices was agreed with the Agency (Minutes of 04 Mar 2014 pre-NDA Meeting) provided that it could be demonstrated that Tween-20 does not impact the binding of BCS Class II and IV drugs to RLY5016S. This was verified

in Study RLY-TR-0155, titled “Effect of Tween-20 on *In vitro* RLY5016S Drug Interactions.”

Each Test Drug was evaluated in 12 replicates in the presence or absence of RLY5016S. The binding reactions were incubated with rotation at 37°C for 3 hours. After incubation, RLY5016S was allowed to settle for 5 minutes, which is sufficient to clear the supernatant of RLY5016S particles for sampling. As shown in Study RLY-TR-0156 titled, “Effect of Filtration Method and Sampling Time on Concentration of Test Drug Not Bound to RLY5016S”, there is no perturbation of the binding equilibrium between RLY5016S and a Test Drug during the 5-minute settling period. Supernatant samples were filtered, collected by centrifugation, and filtrates were analyzed for unbound Test Drug using a qualified method.

As recommended in the FDA draft guidance *Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*, February 2012, results from this study were reported as 90% confidence intervals (CI) about the geometric mean ratio of the recovered Test Drug with and without RLY5016S. An *in vitro* interaction of less than 30% was defined as a clinically insignificant interaction. However, if the CIs fell outside the range of 70 – 143%, it was concluded that an *in vitro* interaction between the Test Drug and RLY5016S had occurred. This is consistent with previous guidance from the Agency (Minutes of 18 Jun 2010 Type C Meeting).

The results from Study RLY-TR-0130 are summarized in Table 6.

Table 6: *In vitro* Drug-Drug Interaction: 90% CI of Test Drug Recovery

Test Drug:	SGF + 0.05% Tween	Acetate Buffer + 0.05% Tween	SIF + 0.05% Tween
Group 1: ~45 – 95% in all three test matrices			
Amlodipine	10.3, 11.4	35.3, 38.0	12.5, 13.6
Cinacalcet	13.0, 13.8	19.0, 19.7	17.7, 19.0
Ciprofloxacin	17.4, 18.6	23.8, 25.8	6.3, 7.5
Levothyroxine[a]	ND	ND	ND
Quinidine	11.9, 13.3	42.2, 44.0	22.9, 25.7
Thiamin	28.6, 28.9	50.0, 51.3	42.2, 43.5
Trimethoprim	35.4, 37.1	54.8, 55.8	27.9, 28.9
Group 2: ~30 – 50% in one test matrix; < 30% in two test matrices			
Clopidogrel[b]	65.5, 66.5	ND	ND
Furosemide	66.7, 67.8	94.0, 95.1	74.5, 84.1
Lithium	92.9, 93.7	88.4, 89.1	56.8, 57.1
Metformin	47.9, 49.9	80.1, 83.5	78.4, 82.0
Metoprolol	71.3, 72.2	84.4, 87.5	67.7, 69.6
Verapamil	50.7, 52.7	88.1, 89.1	77.0, 78.8
Warfarin	65.5, 67.2	91.7, 93.3	95.8, 98.8
Group 3: < 30% in all three test matrices			
Allopurinol	85.2, 86.2	86.2, 94.3	90.0, 98.6
Amoxicillin[c]	ND	97.3, 101.2	94.9, 104.2
Apixaban	75.3, 76.0	97.0, 97.5	97.6, 98.0
Aspirin	100.5, 100.8	98.9, 100.7	99.5, 100.4
Atorvastatin	89.0, 93.4	92.2, 94.4	96.4, 106.0
Cephalexin	88.3, 89.1	92.5, 97.4	102.5, 106.3
Digoxin[c]	ND	107.5, 111.1	100.7, 106.3
Glipizide	72.2, 73.6	96.2, 96.9	97.0, 100.0
Lisinopril	77.5, 78.3	98.3, 103.1	97.0, 100.0
Phenytoin	81.7, 85.2	89.6, 90.7	84.6, 101.7
Riboflavin[d]	93.5, 97.8	ND	94.6, 98.4
Rivaroxaban	71.5, 72.3	92.7, 93.1	94.9, 95.4
Spironolactone	78.0, 79.4	97.2, 99.9	95.2, 98.5
Valsartan	85.5, 87.3	100.9, 101.9	97.1, 98.9

ND = not determined; SGF = simulated gastric fluid, no pepsin; SIF = simulated intestinal fluid, no pancreatin

[a] Levothyroxine is chelated by the calcium of the RLY5016S counterion complex (Synthroid® [levothyroxine sodium tablets, USP] Package Insert, 2013).

[b] Clopidogrel was unstable during binding assay conditions (37°C for 3 hours) in Acetate Buffer + 0.05% Tween-20 and SIF + 0.05% Tween-20 (Report No. RA-024-RPT, Section 3.8.4 and Report No. RA-028-RPT, Section 3.10.2).

[c] Amoxicillin (Hidaka, 2010) and digoxin (Sternson, 1978) are degraded at pH 1.2.

[d] Riboflavin is subject to photolysis in acetate buffer (Ahmad et al., 2014).

Under the conditions of this *in vitro* study, RLY5016S showed a ~45 – 95% interaction with the seven Test Drugs in Table 6, Group 1. All of these drugs are positively charged and, according to the QSPR model, would be expected to bind to RLY5016S. Levothyroxine, which is known to be chelated by calcium (see

Synthroid® [levothyroxine sodium tablets, USP] Package Insert, 2013), becomes insoluble in the presence of the calcium counterion of RLY5016S. Since levothyroxine interacts with calcium, and calcium is a component of RLY5016S, there is a DDI between RLY5016S and levothyroxine.

The seven Test Drugs in Table 6, Group 2 showed a weak (~30 – 50%) interaction (i.e., 90% CIs about the geometric mean ratio of the recovered Test Drug with and without RLY5016S were ~50 – 70%) with RLY5016S in one test matrix and no interaction in the other two matrices.

- Clopidogrel is positively charged under the conditions where it showed weak binding to RLY5016S and neutral under the two conditions where it could not be tested due to instability.

- Lithium is a cation that competes with other matrix cations for binding to the RLY5016 polymer anion. Lithium binding to the polymer increased with increasing pH as the polymer became more negatively charged.

- Although they are positively charged, metoprolol, metformin and verapamil show limited interaction with RLY5016S compared to more strongly basic compounds, which is consistent with the predictions from the QSPR model (see QSPR-RLY-001).

- A very weak interaction, only occurring in SGF + 0.05% Tween-20, was observed between RLY5016S and furosemide and RLY5016S and warfarin. These two Test Drugs are relatively hydrophobic under the conditions of the SGF + 0.05% binding experiments, and the results observed were predicted by the QSPR model because hydrophobicity is also a factor that contributes, albeit weaker than charge (see QSPR-RLY-001).

Finally, no interaction (i.e., < 30%) with RLY5016S was observed with the 14 Test Drugs indicated in Table 6, Group 3 (i.e., 90% CIs about the geometric mean ratio of the recovered Test Drug with and without RLY5016S were > 70%). None of these drugs is positively charged under the conditions of the binding assays; since RLY5016S is a cation binder, the lack of interaction with this group of drugs was expected.

5.1.7.2 QSPR Based Model for Binding of Drugs to RLY5016S

At the pre-NDA Meeting held on 04 March 4 2014, the Agency requested that Relypsa use the data from its *in vitro* binding studies to develop a QSPR based model to predict binding of drugs to RLY5016S. To this end, Relypsa contracted with (b) (4) to build linear regression models for the three *in vitro* systems used to assess binding of Test Drugs to RLY5016S. This work is described in Report No. QSPR-RLY-001.

(b) (4) first computed 121 physical descriptors for the set of Test Drugs for which measured drug binding to RLY5016S was available from Study RLY-TR-0130 (i.e., the training set). Next, a regression model was constructed for each test matrix (i.e., SGF + 0.05% Tween-20, Acetate Buffer + 0.05% Tween-20 and SIF + 0.05% Tween-20) using a subset of these descriptors (chosen via a stepwise model selection). The models were then used to predict binding to RLY5016S for a Test Drug that was not included in the model building experiment. Given the relatively small number of Test Drugs used in the present study, the models were cross-validated using the leave-one-out procedure (cross-validated correlation coefficients were calculated and reported as Q_2). This approach is similar to a published QSPR study that modeled the binding of drugs to the bile sequestrant, colesevelam (Welchol; Walker, 2009). In that study, the authors constructed a multivariate model with the partial least squares regression technique and a total of 11 computed compound descriptors. The leave-one-out cross validation technique was also employed in our study to judge the goodness of fit.

Due to limitations of the computational methodology, thiamin and lithium were excluded from the model building due to methodology limitations (see QSPR-RLY-001). Levothyroxine was excluded from the model building because *in vitro* binding to RLY5016S could not be measured due to precipitation of levothyroxine in the presence of the calcium in the RLY5016S counterion complex (see Report No. RLY-TR-0130).

The three linear regression models developed by (b) (4) enable the prediction of potential RLY5016S drug-drug interactions for various compounds of interest. The models contain a few physically meaningful descriptors that are readily computed from the molecular structure. All three models were able to be built using a relatively small number of computed parameters (six in total for all three models). All of the models yielded a traditional R_2 of ca. 0.7 and a cross validated Q_2 of ca. 0.6. R_2 is the square of the coefficient of multiple correlation; it is a measure of fit and ranges between 0 (no correlation, or predictability) to 1 (explains all variability). An $R_2 = 0.7$ may be interpreted as explaining 70% of the variation in the response variable (i.e., binding to RLY5016S). The remaining 30% can be attributed to unknown variables (likely in the present study) or inherent variability in the system. The small difference between R_2 and Q_2 values suggests that the models are not over-fit and should be predictive for compounds that have not been included in the current study (i.e., $R_2 - Q_2 < 0.3$; Veerasamy, 2011).

The models uncover the factors that have the most influence on binding of drugs to RLY5016S (extent of surface area of acceptor atoms, lipophilicity, functional group counts and energies of various molecular orbitals). The factors that enable a reasonably accurate model to be built can then be interpreted to understand why some compounds interact with RLY5016S, while others do not. The model allows a systematic approach to understanding and/or anticipating potential DDI.

Most of the linear regression terms that were found to be significant related to:

- the computed surface area of acceptor atoms (SAAA1–summation of the total surface area of atoms identified to be hydrogen bond acceptors),

- the ionization potential (IP, energy of the highest occupied molecular orbital), and electron affinity (EA, energy of the lowest unoccupied molecular orbital), and

- the lipophilicity of the compounds as given by X.noncon (which is a count of the number of ring atoms not able to form conjugated aromatic systems, e.g. sp^3 carbon atoms) and QPlogPw (predicted water/gas partition coefficient).

In this study, the surface area of all atoms was computed, and then each atom was classified (as a donor, acceptor, neutral, etc.). The surface area for each type was then partitioned in different ways in an attempt to differentiate various features of each drug. For the molecular orbital terms, the IP and EA (or the energy levels of the HOMO and LUMO molecular orbitals) energies are thought to be related to how easily one molecular system can interact with another. These later quantities can be useful in developing QSPR models, but do not readily lend themselves to simple interpretations. Additional terms that describe the "lipophilicity" of the compounds, such as X.noncon (which is a count of the number of ring atoms not able to form conjugated aromatic systems, e.g. sp3 carbon atoms) and QPlogPw (predicted water/gas partition coefficient) were also included in some of the models. The final term X.amide (number of non-conjugated amide groups) handles a common functional group in a general way to account for its effect on binding to the polymer.

Given the nature of RLY5016S (i.e., a cation binding polymer), the parameters found to be significant in the models are physically reasonable because they describe both the polarity and lipophilicity of the molecules which have long been implicated in small molecule interactions with various proteins and polymers. Table 7 provides a summary of the regression coefficients for each of the three linear regression models developed for RLY5016S DDI.

Table 7: Comparison of the Coefficients for Linear Regression Models

Model	Intercept	SAAA1	IP.eV	EA.eV	QPlogPw	X.noncon	X.amide
SGF	-396.391	NA	46.4826	NA	4.0192	-2.1771	-22.2272
AB	-189.997	0.38176	26.5812	-16.801	NA	-1.60166	NA
SIF	-294.420	0.43712	36.8472	-13.346	NA	-2.21710	NA

AB = Acetate Buffer + 0.05% Tween-20; NA = not applicable; SGF = SGF + 0.05% Tween-20; SIF = SIF + 0.05% Tween-20

Table 7 indicates as the surface area of acceptor atoms is increased, the binding of drugs to the polymer decreases. As the pH increases (SGF < Acetate Buffer <

SIF), RLY5016S becomes more negatively charged, and thus one would expect for drugs with a greater number of acceptor atoms (larger surface area) to bind less favorably. Likewise, the contribution of QPlogPw and X.noncon both suggest that as the compounds become more lipophilic (QPlogPw becomes smaller and X.noncon becomes greater), binding to the polymer may also increase (which, of course, depends on the other parameters such as SAAA1).

The compounds evaluated in Study QSPR-RLY-001 can be categorized as strong binders, non-binders or weak binders to RLY5016S under the current assay conditions. Strong binders to RLY5016S tend to be drugs with basic amines that do not contain balancing polar functionality (such as a carboxylate, cyano, multiple amides) in close proximity to the basic group. Drugs such as amlodipine, cinacalcet, trimethoprim and quinidine are examples that illustrate this class of interacting drugs.

In contrast, a characteristic of nonbinding compounds is the presence of multiple polar functional groups (such as amides, carboxylates, sulfonamides, hydroxyls, etc.). Examples of this class of drugs are amoxicillin, cephalexin, riboflavin, atorvastatin, digoxin, apixaban, furosemide, glipizide, lisinopril, valsartan, rivaroxaban, spironolactone and relatively small, highly polar compounds, such as metformin, aspirin, allopurinol, warfarin and phenytoin. In particular, the inclusion of carboxylates and multiple hydroxyl groups in drugs greatly decreases interaction with RLY5016S. All of the drugs mentioned above possess multiple, highly polar groups that, as the models predict, are essentially nonbinders.

As the number of polar groups decreases, or the lipophilicity of the drug increases, there can be weak binding to RLY5016S. Examples of this include the drugs clopidogrel, metoprolol and verapamil. Drugs in this class tend to be neutral to weakly basic, with limited polar functionality.

It has been shown that the presence of calcium reduces the bioavailability of ciprofloxacin (Stojković, 2014). RLY5016S is a calcium-sorbitol complex of the polymer anion. The interaction between ciprofloxacin and RLY5016S observed *in vitro* could be a combination of interactions between ciprofloxacin and the polymer anion, and ciprofloxacin and calcium. The QSPR models developed do not address the potential interaction between ciprofloxacin and calcium. This may be the reason for the larger than expected difference in the predicted vs. actual binding of ciprofloxacin (see QSPR-RLY-001).

In summary, we have constructed several linear regression models to predict the binding of drugs to RLY5016S based on a small number of readily computed parameters from the molecular structure of the drug alone. Both the accuracy and interpretability of the models suggest that they will be useful for the prediction of drug interactions with RLY5016S for compounds that have not been studied previously.

Reference ID: 3781906

5.1.8 Other Pharmacokinetic Studies

The anion of RLY5016S is a crosslinked polymer of 2-fluoro[redacted]. Fluoride degradation of RLY5016 for Oral Suspension has been demonstrated in stability studies to occur at a rate of approximately[redacted] ppm per month under refrigerated conditions. The driving force for the fluoride elimination is a reaction between the calcium ion and fluoride due to the electropositive nature of calcium and its relative proximity to an electronegative fluorine[redacted]

5.1.8.1 Plasma Fluoride Levels in Rats after Oral Administration of RLY5016 and RLY5016[redacted] (TR 300-07-028)

Prior to conducting human clinical studies, the PK properties of the fluoride degradation product in RLY5016 were explored in rat studies (TR 300-07-028) that measured the absorption of fluoride released from the polymer containing defined amounts of fluoride. In order to calibrate the studies, a pilot study was conducted in nine male rats exposed to NaF and CaF_2 at doses of[redacted] via oral gavage, and plasma fluoride levels were measured over 480 minutes postdose. In the pilot study, peak plasma fluoride levels were attained 15 – 30 minutes postdose, with approximately[redacted] lower fluoride bioavailability (as assessed by AUC) observed in animals given CaF_2 versus NaF. These results were consistent with the available literature, which indicates that CaF_2 ingestion results in[redacted] lower systemic fluoride exposure than NaF ingestion (Trautner, 1987; Whitford, 1991). CaF_2 is nearly insoluble in water[redacted], accounting for its low relative bioavailability compared to NaF[redacted]

In the next series of experiments, RLY5016 (the[redacted] and RLY5016[redacted] (the[redacted]), each containing various fluoride levels, were administered to rats via oral gavage, and systemic exposure to fluoride was assessed over 480 minutes postdose. The dose groups in these experiments were expressed as fluoride dose per kg animal weight (in mg/kg). The mean plasma fluoride concentration-time profiles for animals receiving oral doses of RLY5016 and RLY5016[redacted] at various fluoride dose levels are shown in Figure 9 and Figure 10.

Figure 9: Mean Plasma Fluoride Concentrations after Administration to Rats of RLY5016 Containing a Range of Fluoride Levels

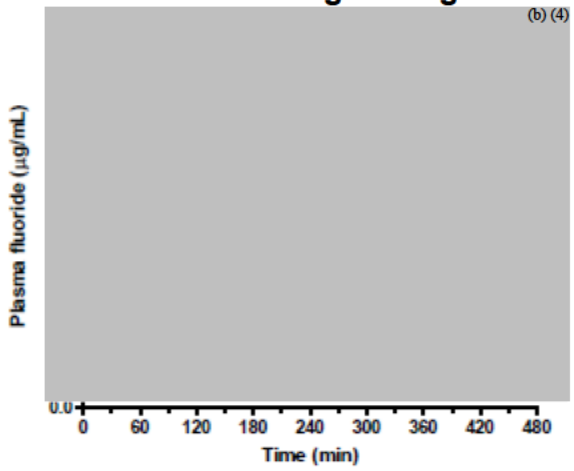
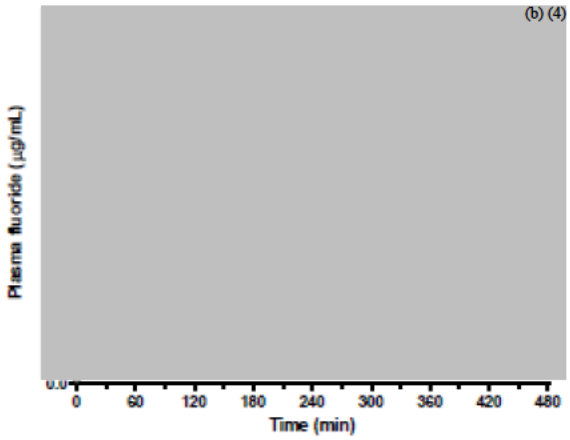


Figure 10: Mean Plasma Fluoride Concentrations after Administration to Rats of RLY5016 (b) (4) Containing a Range of Fluoride Levels



Results from these experiments demonstrated that fluoride bioavailability from RLY5016 was lower than fluoride bioavailability from RLY5016 (b) (4). Peak plasma fluoride levels occurred at 30 minutes after administration of RLY5016 (b) (4) and 30 – 60 minutes after administration of RLY5016. When similar fluoride doses were compared (e.g., (b) (4) RLY5016 (b) (4) versus (b) (4) RLY5016), fluoride AUC values were approximately (b) (4) times lower in animals dosed with RLY5016 compared with RLY5016 (b) (4) (Table 8). Similarly, fluoride C_{max} values were approximately (b) (4) lower with RLY5016 versus RLY5016 (b) (4) (Table 8). These data demonstrate that dosing with the RLY5016 polymer anion that has a calcium counterion generates fluoride absorption that is substantially lower than that seen with the polymer anion (b) (4) with a sodium counterion.

Table 8: Pharmacokinetic Parameters for Fluoride after Administration of RLY5016 and RLY5016 (b) (4) to Rats

Fluoride Dose (mg/kg)	$AUC_{(0\text{-}690\ min)}$ (mcg • min/mL)	C_{max} (mcg/mL)
RLY5016		
	(b) (4)	
RLY5016Na		
	(b) (4)	

AUC = area under curve; C_{max} = maximum plasma concentration; mcg = microgram
Source: adapted from TR 300-07-028, Table 3 and Table 4
Comparable fluoride doses shown in bold

5.1.9 Discussion and Conclusions

The extent of RLY5016 bioavailability has been assessed in rats and dogs using ^{14}C-labeled RLY5016 beads. Plasma, fecal, and urinary monitoring was conducted to assess possible absorption and distribution of the drug. Analysis showed that 0.004% and 0.002% of the administered radiolabel was present in plasma in the rat and dog, respectively. Quantitative whole body autoradiography in rats did not detect any radiolabel outside the GI tract. Recovery of the radiolabel in feces and urine was 84.1% and 0.15%, respectively, in rats and 99.9% and < 0.1%, respectively, in dogs. The lower fecal recovery observed in rats was attributed to difficulty collecting and homogenizing rat feces. The amount of radiolabel recovered in the urine of both species was consistent with the level of unincorporated radiolabeled crosslinker present in the radiolabeled polymer, and the majority of the unincorporated radiolabel ((b) (4) %) was found in a single peak that co-eluted with the (b) (4) used in the synthesis of the bead. Therefore, studies in both the rat and the dog demonstrated the nonabsorbed nature of the polymer and its lack of systemic bioavailability.

RLY5016 is stable and does not degrade during passage through the GI tract. The physical stability of the RLY5016 beads during transit through the GI tract was demonstrated by recovering the product from fecal samples collected from human subjects (RLY-TR-0054). The beads recovered from feces remain as intact, (b) (4) spheres. The metabolic stability of the beads was demonstrated by analysis of cation content of the recovered RLY5016 beads: at all doses, the sum of the major physiologic cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) bound to the recovered (b) (4) beads equaled the amount of calcium ion bound to beads that had not passed through the GI tract. Thus, RLY5016 is not

metabolized or changed by GI passage, as loss of cation-binding units due to such metabolism would be reflected in altered cation content.

As agreed with the Agency at the pre-NDA Meeting (Minutes of 04 Mar 2014 pre-NDA Meeting), a biologically-relevant *in vitro* test system was used to evaluate potential interactions between RLY5016S and a set of orally administered compounds commonly used in the target patient population for RLY5016 for Oral Suspension. In addition, a QSPR based model was developed that reliably predicts the binding of drugs to RLY5016S. Binding to RLY5016S can be predicted based on a small number of readily computed parameters from the molecular structure of the potentially interacting drug alone. These are the (1) computed surface area of hydrogen bond acceptor atoms, (2) ionization potential and electron affinity, and (3) lipophilicity of the potentially interacting drug. Given the nature of RLY5016S (i.e., a cation binder), the parameters found to be significant are physically reasonable because they describe both the polarity and lipophilicity of the molecules, both of which have long been implicated in small molecule interactions with various proteins and polymers. The results of the *in vitro* DDI study and the QSPR modeling are adequate to propose specific dosing recommendations in the label to avoid untoward drug interactions with RLY5016 for Oral Suspension.

Finally, a rat model was used to test the hypothesis that minimal fluoride absorption would result from the oral administration of RLY5016, due to the presence of fluoride in RLY5016 as a CaF_2 (b) (4), which has low solubility and bioavailability. Rats were orally administered RLY5016 (b) (4) and RLY5016 such that they received a range of dose levels of fluoride from each compound, and the PK of fluoride was subsequently assessed in plasma. The results demonstrated that at comparable doses, RLY5016 gave a (b) (4) lower C_{max} and a (b) (4) lower fluoride AUC compared to RLY5016 (b) (4). These data demonstrate that dosing with the RLY5016 polymer anion that has a calcium counterion generates fluoride absorption that is substantially lower than that seen with the polymer anion (b) (4) with a sodium counterion.

5.1.10 References

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5.2 Toxicokinetics

As RLY5016S is not absorbed from the gastrointestinal tract, toxicokinetic measurements were not made in the toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

RLY5016S is not absorbed from the gastrointestinal tract. Consistent with other polymeric drugs, minimal toxicity was expected; therefore, single dose (acute) toxicity studies were not conducted.

6.2 Repeat-Dose Toxicity

Repeat-dose toxicity studies were performed in rats and dogs. These are summarized in Table 1 and are summarized below. Animals in the *in vivo* studies were administered oral doses of the drug substance, either as a dietary admixture, via oral gavage in carboxymethylcellulose (CMC) or in capsules. As part of the toxicity studies, the stability of the polymer in the dosing formulations was assessed using comparisons of the total calcium content of the formulated dose over time.

Table 1: Repeat-dose Toxicity Studies

Report Number	Route of Admin	Species	Gender: No./Group	Duration	Target Doses	NOAEL
TR 350-07-009	Oral (diet admixture)	Rat	M:5 F:5	2 weeks plus 2-week recovery	0, 4, 8, 10, 15 g/kg/day	> 17.6 g/kg/day
TR 350-07-010	Oral (diet admixture)	Rat	0, 15 g/kg/day: M:15, F:15 4, 10 g/kg/day: M:10, F:10	4 weeks, plus 2-week recovery	0, 4, 10, 15 g/kg/day	ND[a]
TR 350-07-018	Oral (diet admixture)	Rat	0, 15 g/kg/day: M:15, F:15 4, 10 g/kg/day: M:10, F:10	4 weeks, plus 2-week recovery	0, 4, 10, 15 g/kg/day	> 15 g/kg/day
TR 350-09-001	Oral (diet admixture)	Rat	0, 5 g/kg/day: M:20, F:20 1, 2.5 g/kg/day: M:15, F:15	26 weeks, plus 4-week recovery	0, 1.0, 2.5, 5.0 g/kg/day	> 5 g/kg/day
TR 350-07-011	Oral (capsule)	Dog	Ph 1: M:1, F:1 Ph 2: M:2, F:2	Ph 1: 1 day Ph 2: 10 days	Ph 1: 2, 4, 7 g/kg/day Ph 2: 7 g/kg/day	> 7 g/kg/day
TR 350-07-015	Oral (capsule)	Dog	Control & high-dose: M:5, F:5 Low- & mid-dose: M:3, F:3	4 weeks, plus 2-week recovery	0, 1, 3, 7 g/kg/day	NOEL = 3 g/kg/day[a]
TR 350-07-019	Oral (capsule)	Dog	Control & high-dose: M:5, F:5 Low- & mid-dose: M:3, F:3	4 weeks, plus 2-week recovery	0, 1, 3, 7 g/kg/day	> 7 g/kg/day
TR 350-10-001	Oral (capsule)	Dog	Control & high-dose: M:5, F:5 Low- & mid-dose: M:3, F:3	39 weeks, plus 4-week recovery	0, 1, 2, 3.75 g/kg/day	3.75 g/kg/day

F = female; M = male; ND = not determined; NOAEL = no observed adverse effect level; NOEL = no observed effect level; Ph = phase; TR = Technical Report

[a] (b) (4)

Studies **TR 350-07-018** (4 week) and **TR 350-09-001** (26 week) were the pivotal rat toxicology studies, while **TR 350-07-019** (4 week) and **TR 350-10-001** (39 week) are the pivotal dog studies.

The pivotal 4 week rat and dog studies were previously reviewed at the IND submission, and summarized below. The 26 and 39 week studies are reviewed herein.

6.2.1 2-Week Dose Range-Finding Repeat-Dose Toxicity Study in Rats (TR 350-07-009)

The purpose of this study (TR 350-07-009, titled "2-Week Dietary Dose Range-Finding Toxicity Study with RLY5016 in Rats with a 2-Week Recovery") was to evaluate the palatability of dietary admixes containing RLY5016 (i.e., polymer anion (b) (4) with calcium counterion), as well as the potential toxicity of the test article when administered daily in the diet to rats for 2 weeks. In addition, the study assessed the reversibility, persistence and delayed occurrence of any effects after a 2-week recovery.

Groups of five Crl:CD(SD) rats per sex were administered RLY5016 orally via diet admixture at target dose levels of 0, 4, 8, 10 and 15 g/kg/day for 14 days. After the 14-day dosing period, two rats per sex per group were placed on a 14-day observation period. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption and clinical pathology results.

The following mean doses were achieved, as calculated from food consumption and body weight data: 0, 4.3, 8.8, 11.3 and 18.2 g/kg/day for males and 0, 4.1, 8.4, 11.0 and 17.0 g/kg/day for females.

All rats survived to scheduled sacrifice. There were no test article-related effects in any of the parameters examined, with the exception of dose-related increases in mean food consumption in rats administered target dose levels of 10 and 15 g/kg/day RLY5016 during the dosing phase. These increases likely reflect the decreased nutritive aspects of the diet (on a weight/weight basis) resulting from the high concentrations of test article in the diet and, as such, were not considered adverse. There were no adverse test article-related clinical pathology findings or anatomic pathology findings (macroscopic and microscopic observations and/or organ weight changes) observed after the dosing or recovery phases.

In conclusion, dietary admixtures of RLY5016 were palatable up to and including a target dose of 15 g/kg/day. In this study, the NOAEL for RLY5016 when provided in dietary admixes to rats for 2 weeks was > 17.6 g/kg/day.

6.2.2 4-Week Repeat-Dose Toxicity Study in Rats (TR 350-07-010)

The purpose of this study (TR 350-07-010, titled "RLY5016: 4-Week Dietary Toxicity Study in Rats with a 2-Week Recovery") was to evaluate the toxicity of RLY5016 (i.e., polymer anion (b) (4) with calcium counterion) when administered daily in the diet to rats for at least 4 weeks and to assess the reversibility, persistence or delayed occurrence of any effects after a 2-week recovery. This study was conducted with a RLY5016 lot (Lot No. CHTL00263) that was produced (b) (4) (TR 350-07-010,

Appendix 3). Consequently, it was strongly suspected that the toxicological findings in this study were related to [redacted] in the final drug substance. [redacted] is not used in the current drug substance manufacturing process. Male and female Crl:CD(SD) rats were assigned to four groups (15 rats/sex/group in the control and high-dose groups and 10 rats/sex/group in the low- and mid-dose groups). The control group (Group 1) received the diet only (no RLY5016). The test groups (Groups 2, 3 and 4) received a target daily dose of RLY5016 at 4, 10 or 15 g/kg/day, respectively, mixed with the chow. Ten (10) rats/sex/group were designated for terminal sacrifice after 4 weeks of treatment; an additional five rats/sex in Group 1 and four rats/sex in Group 4 were designated for recovery sacrifice after a 2-week non-treatment phase following dosing. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, ophthalmic examinations, and clinical and anatomic pathology results.

Due to decreased food consumption (particularly at the mid- and high-dose levels), the actual mean doses achieved for Groups 2, 3 and 4 were 4.0, 7.5 and 8.9 g/kg/day for the males and 4.1, 8.0 and 11.8 g/kg/day for the females, respectively.

Two high-dose rats were sacrificed in moribund condition: one male on Day 10 and one female on Day 16. Corresponding clinical signs included hypoactivity and hunched appearance. Treatment-related clinical signs were noted primarily in the high-dose rats and included thin appearance, rough hair coat and squinted eyes. It was speculated that the presence of the test article in the diet adversely affected the palatability of the diet based on the dose-related decrease in food consumption, which was significantly lower for the low- (88%), mid- (68%) and high-dose (51%) males and the mid- (97%) and high-dose (76%) females. There was a corresponding decrease in mean body weight gain. After 4 weeks of treatment, the mean body weights for low-, mid- and high-dose rats were 82%, 66% and 48% of control for the males and 94%, 84% and 73% of control for the females, respectively. During the 2-week recovery phase, there was a marked increase in food consumption and body weight gain in the high-dose rats as compared to the dosing phase.

Treatment-related alterations in the clinical pathology data were observed primarily in the mid- and high-dose rats, with a few changes seen in individual low-dose rats. The values for these parameters were generally comparable between control and high-dose rats following 2 weeks of recovery. Following 4 weeks of treatment, microscopic findings ascribed to RLY5016 in the diet were present in the kidney, femur and sternum bones and marrow, stomach and testes of the low- and/or high-dose rats. Secondary changes likely relating to stress and/or poor condition included atrophy in the prostate, seminal vesicle, uterus, salivary gland and pancreas, and lymphoid depletion/necrosis in the spleen and thymus in the high-dose rats. Following a 2-week recovery period, treatment-related kidney, stomach, testes, femur and sternum bones and marrow changes

were noted. There was a decrease or absence of all of the secondary microscopic changes that were considered related to poor condition or stress in the high-dose recovery rats.

In conclusion, a NOAEL for RLY5016 (Lot No. CHTL00263) could not be determined following 4 weeks of treatment based, in part, on microscopic findings in the kidney and stomach of rats that received diets containing RLY5016. Based on a lack of similar findings in the 2-week dose range-finding study (TR 350-07-009), it was strongly suspected that the findings in this study were related to a contaminant (i.e., (b) (4)) present in this lot of RLY5016. The presence of (b) (4) could also account for the decreased food consumption (i.e., decreased palatability) noted in this study.

6.2.3 4-Week Repeat-Dose Toxicity Study in Rats (TR 350-07-018)

Due to the unexpected findings in the initial 4-week rat toxicity study (TR 350-07-010), the study was repeated with a RLY5016 lot for which (b) (4) (Lot No. CHZC009681), rather than (b) (4), which was used in the previous 4-week rat toxicity study. The purpose of this study (TR 350-07-018, titled "RLY5016: 4-Week Dietary Toxicity Study in Rats with a 2-Week Recovery") was to evaluate the toxicity of RLY5016 (i.e., polymer anion (b) (4) with calcium counterion) when administered daily in the diet to rats for at least 4 weeks and to assess the reversibility, persistence or delayed occurrence of any effects after a 2-week recovery.

Male and female Crl:CD(SD) rats were assigned to four groups (15 rats/sex/group in the control and high-dosage groups and 10 rats/sex/group in the low- and mid-dosage groups). The control group (Group 1) received the diet only (no RLY5016). The test groups (Groups 2, 3 and 4) received a target daily dose of RLY5016 at 4, 10 or 15 g/kg/day, respectively, mixed with the chow. Ten rats/sex/group were designated for terminal sacrifice after 4 weeks of treatment; an additional five rats/sex in Group 1 and four rats/sex in Group 4 were designated for recovery sacrifice after a 2-week non-treatment phase following dosing. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, ophthalmic examinations, and clinical and anatomic pathology results.

In this admixture repeat-dose study, no adverse effect on food consumption was observed; the mean dosage levels of RLY5016 achieved over the 4-week period for Groups 2, 3 and 4 were 3.4, 8.6 and 12.8 g/kg/day for the males and 3.7, 9.0 and 13.9 g/kg/day for the females, respectively.

Assessment of toxicity was based on mortality, clinical signs, body weight changes, food consumption, ophthalmic examinations and clinical and anatomic pathology. In this study, there were no RLY5016-related mortalities, clinical or

ophthalmic observations or adverse effects on body weight or body weight gain during the dosing or recovery phase. Significant dosage-related increases in mean food consumption were noted in rats given target dosage levels of 4, 10 and 15 g/kg/day RLY5016; these results are similar to those observed in the 10 and 15 g/kg/day dosage groups in the 2-week study, TR 350-07-009. These increases likely reflect the decreased nutritive aspects of the diet (on a weight basis) due to the high concentrations of test article in the diet and, as such, were not considered adverse. The mean food consumption in the high-dose group rats was not significantly different from control rats during the recovery phase. There were no RLY5016-related effects on hematologic parameters. Total protein and albumin were significantly lower in high-dose group female rats and urinary calcium excretion was higher in high-dose group rats of both sexes. The elevation in urinary calcium excretion was not unexpected since calcium is the counterion associated with the polymer in RLY5016. With the exception of urinary calcium excretion in the high-dosage group females, total protein, albumin and calcium excretion were similar between control and 15 g/kg/day rats at recovery Day 15. There was no microscopic correlate to these laboratory value differences, and the mean values were similar to control values by recovery Day 15; therefore, the changes were not considered adverse. There were no RLY5016-related alterations in organ weight, macroscopic or microscopic findings in rats sacrificed after the dosing or recovery phases.

In summary, rats administered RLY5016 (target dosages of 0, 4, 10 and 15 g/kg/day) daily in the diet for 4 weeks showed no RLY5016-related changes in mortality or clinical/ophthalmic observations and no adverse effects on body weight, body weight gain, food consumption or clinical pathology and no macroscopic or microscopic changes. Therefore, the NOAEL for RLY5016 in the study was > 15 g/kg/day.

Reference ID: 3781906

6.2.4 26-Week Dietary Toxicity Study with RLY5016S in Rats

Study title: 26-Week Dietary Toxicity Study with RLY5016S in Rats with a 4-Week Recovery Period

Study no.:	TR 350-09-001
Study report location:	NDA 205739 SDN 0001
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	12/16/2008
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	See chart below

The duration of use for the lots of RLY5016S used in this study was not provided in the report. These data are provided below.

Study Week(s)	RLY5016S Lot Number	Comments
Weeks 1 – 3	CHZE204947	
Week 4	CHZE204947 CHZE205749	Both lots were used in this weekly preparation.
Weeks 5 – 22	CHZE205749	
Week 23	CHZE205749 LTFLAA1003	Both lots were used in this weekly preparation.
Weeks 24 – 26	LTFLAA1003	

Key Study Findings

There was no RLY5016S-related mortality. There were no dose-dependent clinical signs of toxicity, ophthalmic findings, or RLY5016S-effects on mean body weight or mean body weight change, clinical pathology, organ weight changes, macroscopic observations, or microscopic findings. Based on the lack of adverse test article related findings in the study data, the NOAEL for RLY5016S administered daily in the diet to rats for 26 weeks was determined to be > 5.0 g/kg/day (expressed as RLY5016).

There was a RLY5016S-related increase in food consumption at 2.5 (primarily females) and 5.0 g/kg/day for both sexes when compared to control during the dosing phase and during the first two weeks of the recovery phase for 5.0 g/kg/day males. These increases in food intake likely reflected the decreased nutritive aspects of the diet (on a weight basis) due to the high concentrations of test article in the diet

Methods

Doses:	1, 2.5 and 5.0 g/kg/day
Frequency of dosing:	daily
Route of administration:	Dietary admixture
Dose volume:	na
Formulation/Vehicle:	na
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	15
Age:	7 weeks
Weight:	178 to 228 g for males and 154 to 203 g for females
Satellite groups:	5/sex for recovery, Control and HD only
Unique study design:	no
Deviation from study protocol:	The study deviations neither affected the overall interpretation of study findings nor compromised the integrity of the study.

Observations and Results

Mortality

Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress; findings were recorded as they were observed.

There was no RLY5016S-related mortality. A moribund male dosed at 2.5 g/kg/day (B49131) was euthanized on Day 122 of the dosing phase. The cause of this animal's moribundity was a skull fracture in the region of the facial bones.

Clinical Signs

Daily cageside observations were performed during the dosing and recovery phases at the a.m. room check; abnormal findings were recorded. Detailed observations were performed at least once during the predose phase, before dosing on Dosing Phase Day 1, weekly thereafter, and on the day of scheduled sacrifice.

There were no dose-dependent clinical signs of toxicity during either the dosing or recovery phase.

Body Weights

Body weight was recorded at least once during the predose phase, before dosing on Dosing Phase Day 1, and weekly thereafter.

There were no RLY5016S-related changes in male mean body weight or mean body weight change during either the dosing or recovery phase, with the exception of a decrease in mean weight gain for Week 24 at 2.5 and 5.0 g/kg/day. For females, a significant decrease in mean weight change for Dosing Phase Week 10 followed by a significant increase for Dosing Phase Week 11 of the dosing phase was noted when compared with control.

Reference ID: 3781906

Feed Consumption
Measured weekly during the dosing and recovery phases. The last week of food consumption for the treatment phase was measured at Dosing Phase Week 27 (Day 183). The first week of untreated food consumption for the recovery phase was a 6-day food consumption measured at Recovery Phase Week 1 on Day 7.

There was a RLY5016S-related increase in food consumption at 2.5 and 5.0 g/kg/day when compared with control. These increases generally reached statistical significance at 5.0 g/kg/day for both sexes and were sporadically significant at 2.5 g/kg/day (primarily in females) over the dosing phase of the study. During the first 2 weeks of the recovery phase, significant increases in food consumption were noted for 5.0 g/kg/day males, decreasing to values similar to control for the final 2 weeks of recovery.

Female rats previously dosed at 5.0 g/kg/day had mean food consumption similar to control for the entire recovery phase. These increases in food intake likely reflected the decreased nutritive aspects of the diet (on a weight basis) due to the high concentrations of test article in the diet and were anticipated based on an earlier study since the 2.5 and 5.0 g/kg/day dosages approached or exceeded nutritional recommendations of additives to diets not to exceed 5% of the dietary composition.

Ophthalmoscopy
Performed on Predose Phase Day 4, on Dosing Phase Day 182, within 7 days of the terminal necropsy, and on Recovery Phase Day 27 within 7 days of the final necropsy. Animals were examined by a staff veterinarian using an indirect ophthalmoscope.

There were no predose ophthalmology findings. During the dosing phase, pale linear streaks were noted in one control male and in one female at each of the treated dose levels. During the recovery phase, pale linear streaks were noted in one control male and were absent in control and 5.0 g/kg/day females. These findings in the groups administered RLY5016S were unrelated to treatment since no dose response was observed.

Hematology
Parameters:

differential blood cell count	mean corpuscular volume	mean corpuscular hemoglobin	mean corpuscular hemoglobin concentration	white blood cell (leukocyte) count
hematocrit	platelet count	hemoglobin	differential blood cell count	blood smear
reticulocyte count	red blood cell (erythrocyte) count			

Reference ID: 3781906

Coagulation parameters:
prothrombin time, activated partial thromboplastin time

Clinical Chemistry
Blood samples were collected for hematology, coagulation, and clinical chemistry from fasted animals (all surviving rats) via a jugular vein during Dosing Phase Week 13 (Day 88) and at scheduled sacrifices during Dosing Phase Week 27 (Day 184) and Recovery Phase Week 5 (Day 29).

Parameters:

glucose	globulin	albumin	calcium
chloride	potassium	sodium	urea nitrogen
urea nitrogen	total protein	cholesterol	triglycerides
creatinine	alkaline phosphatase	total bilirubin	inorganic phosphorus
albumin/globulin ratio	alanine aminotransferase	aspartate aminotransferase	gamma glutamyltransferase
serum protein electrophoresis			

Urinalysis
Urine samples were collected from the fasted animals overnight chilled before scheduled blood collection intervals for urinalysis and urine chemistry.

Parameters:

volume	specific gravity	protein	pH
glucose	ketones	bilirubin	urobilinogen
sodium	potassium	chloride	blood
sodium excretion	potassium excretion	chloride excretion	
appearance (clarity and color)	microscopic examination of sediment		

RLY5016S administration had no obvious effects on clinical pathology test results during either the dosing or recovery phase. Several statistically significant differences in clinical pathology parameters were observed between the control and treated groups, but they were not considered obvious effects of RLY5016S because they were of small magnitude, often transient, inconsistent between the sexes, and/or lacked a dose-dependent response.

Gross Pathology
After at least 26 weeks of treatment (Dosing Phase Week 27, Day 184), all surviving rats designated for scheduled sacrifice were fasted overnight (15 rats/sex/group except for 14 males in Group 3) then anesthetized with sodium pentobarbital, exsanguinated, and necropsied. Terminal body weights were recorded

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Organ Weights
Protocol-specified organs (when present) were weighed at each scheduled sacrifice; paired organs were weighed together.

adrenal (2)	brain	epididymis (2)	prostate
salivary gland [mandibular (2)]	spleen	heart	kidney (2)
liver	lung	ovary (2)	pituitary gland
testis (2)	thymus	thyroid (2 lobes) with parathyroid	uterus

No treatment-related organ weight changes were noted at the end of the dosing or recovery phase. The few statistically significant organ weight changes lacked a macroscopic and/or microscopic correlation and were attributed to normal biological variation.

Histopathology
The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin, unless otherwise indicated below.

adrenal (2)
aorta
brain
cecum
cervix
colon
duodenum
epididymis (2)[a]
esophagus
eye (2)[a]
femur with bone marrow (articular surface of the distal end)
Harderian gland[a]
heart
ileum
jejunum
kidney (2)
lesions
liver
lung with large bronchi
lymph node (mandibular)
lymph node (mesenteric)
mammary gland (females)
optic nerve (2)[a]
ovary (2)
pancreas
Peyer's patch (gut associated lymphoid tissue)
pituitary gland
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle
skeletal muscle (thigh)
skin/subcutis
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)[a]
thymus
thyroid (2 lobes) with parathyroid
tongue
trachea
urinary bladder
uterus
vagina

a Preserved in modified Davidson's fixative.

Adequate Battery
yes

Peer Review
There was no mention of peer review. This may be related to the lack of any microscopic findings.

Histological Findings

There were no RLY5016S-related macrocopic or microscopic findings.

Toxicokinetics na

Dosing Analysis
Results of concentration verification show that diet preparations were generally within acceptable limits and suitable for dose administration in rats during Weeks 1, 2, 3, 4, 5, 6, 8, 9, 13, 18, and 26 of the dosing phase.

6.2.5 Escalating-Dose Range-Finding Study in Dogs (TR 350-07-011)

The purpose of this study (TR 350-07-011, titled “Escalating Oral Capsule Dose Range-Finding Toxicity Study with RLY5016 in Dogs”) was to evaluate the toxicity, the maximum tolerated dose or the maximum feasible dose, of RLY5016 (i.e., polymer anion complexed with calcium counterion) when given as escalating doses (Phase 1) and for 10 consecutive days (Phase 2) to purebred male and female beagle dogs.

In Phase 1, one male and one female dog were administered escalating doses of RLY5016 via oral capsule twice daily (approximately 4 hours apart) on Day 1 (2 g/kg/day), Day 4 (4 g/kg/day) and Day 7 (7 g/kg/day). In Phase 2, two male and two female dogs were administered 7 g/kg/day RLY5016 via oral capsule twice daily (approximately 4 hours apart) for 10 days. In both phases, dogs were evaluated for mortality, clinical signs, body weight, food consumption, and clinical and anatomic pathology (Phase 2 only).

In Phase 1 of the study, both dogs survived to scheduled termination on Day 9. Unformed feces were noted in the male after each escalating dose (Days 2, 6 and 8). Body weight and food consumption were not affected, although food consumption was slightly lower for both dogs on Day 8. There were no RLY5016-related changes in clinical pathology parameters.

In Phase 2 of the study, all dogs survived to scheduled necropsy on Day 11. Dry and granular feces were noted in one male and both females near the end of the study. Liquid and mucoid feces were also observed in one female on Day 9.

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These clinical observations were considered treatment-related but not adverse. The body weight of one female was decreased by 9%, with significantly reduced mean food consumption during Days 1 – 11. There were no remarkable changes in individual body weight and food consumption in the other dogs in Phase 2. There were no test article-related clinical pathology, macroscopic or microscopic findings.

Dogs administered 7 g/kg/day for 10 days were given 4 – 6 (size 12) gelatin capsules, capable of holding 4.2 – 5.5 g of RLY5016 per capsule, twice daily. This number of capsules was found to be the limit that could be administered to dogs. Therefore, a maximum feasible dosage of 7 g/kg/day was reached in the study. The maximum tolerated dosage of RLY5016 in beagle dogs was determined to be > 7 g/kg/day.

6.2.6 4-Week Repeat-Dose Toxicity Study in Dogs (TR 350-07-015)

The purpose of this study (TR 350-07-015, titled "4-Week Oral Capsule Toxicity Study with RLY5016 in Dogs with a 2-Week Recovery") was to evaluate the toxicity of RLY5016 when administered at 1, 3 and 7 g/kg/day daily via oral (size 12) gelatin capsule for 4 weeks to purebred male and female beagle dogs, and to assess the reversibility, persistence or delayed occurrence of any effects after a 2-week recovery period. This study was conducted with RLY5016 lots (Lot Nos. CHTL00261 and CHTL00262) that were produced (b) (4) (TR 350-07-015, Appendix 3). Consequently, it was strongly suspected that the toxicological findings in this study were related to (b) (4) in RLY5016. (b) (4) is not used in the current drug substance manufacturing process.

Dogs (N = 32) were assigned to one of four groups (5/sex/group in Groups 1 and 4; 3/sex/group in Groups 2 and 3). Group 1 received empty capsules (control). Groups 2, 3 and 4 received oral capsules containing 1, 3 and 7 g/kg/day RLY5016, respectively. All dogs were dosed twice daily (approximately 4 hours apart) for at least 4 weeks (dosing phase). Dogs designated for recovery sacrifice underwent 2 weeks of recovery following dose administration. Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, ophthalmic findings, electrocardiographic findings, and clinical and anatomic pathology.

All dogs survived to scheduled termination. There were no remarkable clinical signs in dogs in the low-dosage (1 g/kg/day) and mid-dosage (3 g/kg/day) groups. Remarkable clinical signs were observed in the majority of dogs in the high-dosage (7 g/kg/day) group after 8 days of dosing; these included dehydration, thin appearance, hypoactivity and granulated and/or dry feces. Based on these clinical signs, as well as significantly decreased body weight on Day 9, dosing in the high-dose group ceased until Day 15 when dosing was resumed at 5 g/kg/day. Higher frequencies of vomiting and granulated and/or dry

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feces were observed in dogs in the high-dose group, and two females in this group were noted to be dehydrated and thin. These clinical signs were not remarkable during the recovery phase.

There were no RLY5016-related effects on body weight, body weight change or food consumption in dogs dosed up to 3 g/kg/day. Significantly reduced body weights and body weight gain were noted on Day 8 in dogs receiving 7 g/kg/day RLY5016. During the dose cessation period, body weights increased in this group, indicating recovery. During the resumed dosing phase (5 g/kg/day), body weights increased or remained unchanged (with the exception of two females). The mean body weight of dogs in the high-dosage group increased during the recovery phase and the effect on food consumption correlated with body weight changes.

No RLY5016-related ophthalmic or electrocardiographic findings were noted.

Reversible, RLY5016-related, effects were noted in the clinical pathology data at Days 30 and/or 43. These included slightly lower erythrocyte count, hemoglobin and hematocrit values and reticulocyte counts in a few dogs in the high-dosage group and slightly lower lymphocyte counts in two female high-dose group dogs. Reversible changes in the serum chemistry data included slightly higher alanine aminotransferase activity in a few dogs in the high-dosage group and slightly lower total protein, albumin and calcium values seen in a few males and most females in this group. A treatment-associated decrease in inorganic phosphorous was also observed in a few high-dosage group dogs.

There were no RLY5016-related macroscopic findings in the study. RLY5016-related microscopic changes were confined to the liver and thymus in high-dosage group dogs. These changes consisted of minimal to slight vacuolation of hepatocytes and minimal to moderate thymic involution, both of which were slightly more prevalent in the females and did not persist during recovery.

In summary, beagle dogs were administered RLY5016 twice daily via oral capsules for at least 4 weeks at 0, 1, 3 and 7 (reduced to 5) g/kg/day. RLY5016-related clinical signs, reduction in body weight and food consumption, clinical pathology changes and microscopic pathology changes were noted in the high dose group. The NOEL in this study was 3 g/kg/day RLY5016.

Given that analysis of the RLY5016 lots used in this study revealed (b) (4) , a second 4-week dog toxicity study was conducted (TR 350-07-019).

6.2.7 4-Week Repeat-Dose Toxicity Study in Dogs (TR 350-07-019)

After it was discovered that the previous 4-week repeat-dose toxicity study in dogs had been conducted with RLY5016 containing (b) (4), this

additional study (TR 350-07-019, titled “4-Week Oral Capsule Toxicity Study with RLY5016 in Dogs with a 2-Week Recovery”) was performed. The objectives and design were the same as those of the previous 4-week study in dogs; however, this study used a drug substance lot (Lot No. CHZC009681) that was produced by incorporating (b) (4).

Male and female purebred beagle dogs were administered 0, 1, 3 or 7 g/kg/day RLY5016 for 4 weeks. A total of 32 animals were administered oral capsules twice daily. The low- and mid-dosage groups (1 and 3 g/kg/day RLY5016, respectively) comprised 3 dogs/sex/group and the control (no RLY5016) and high-dose group (7 g/kg/day RLY5016) comprised 5 dogs/sex/group. After 4 weeks of treatment, 3 dogs/group/sex were sacrificed. The remaining two dogs/sex in the control and high-dosage groups were sacrificed after 2 weeks of recovery. Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, ophthalmic findings, electrocardiographic findings and clinical and anatomic pathology.

There were no RLY5016-related mortalities, or effects on ophthalmologic, electrocardiographic or clinical pathology parameters, organ weights, or macroscopic or microscopic pathology.

There were no RLY5016-related effects on body weight or food consumption, with the exception of one female dog in the high-dose group. This dog exhibited body weight loss due to a reduced food consumption during Week 1 of the study. Dosing of this dog was discontinued on Day 8 and resumed on Day 11. Since the body weight loss was rapidly reversible and occurred without pathological findings, the weight loss and reduced food consumption in this dog were considered treatment-related but not adverse. Clinical observations were limited to several incidences of granular and dry feces noted in the dogs in the mid- and high-dosage groups; these findings were considered related to treatment but not adverse. The NOAEL in this study was > 7 g/kg/day.

6.2.8 39-Week Repeat-Dose Toxicity Study in Dogs (TR 350-10-001)

Study title: 39-Week Oral (Capsule) Toxicity Study with RLY5016S in Dogs with a 4-Week Recovery Period

Study no.: TR 350-10-001

Study report location: NDA 205739 SDN 0001

Conducting laboratory and location: (b) (4)

Date of study initiation: 12/17/2008

GLP compliance: The study design was based on Japanese Ministry of Health, Labor and Welfare Guidelines for Toxicity Studies of Drugs (Notification No. 88, Pharmaceutical Affairs Bureau, 10 August 1993, and Notification No. 655, 05 April 1999) and on Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Section 4, Health Effects, No. 409 (1998).

QA statement: yes

Drug, lot #, and % purity:

Test Article[a]	Lot No.	Storage	Purity	Retest Date
RLY5016S	LTFLAA1001 LTFLAA1003 CHZE206020 CHZE205751 CHZE205152	Refrigerated, protected from light, and stored on desiccant	(b) (4)	(b) (4)

Key Study Findings

RLY5016S-related clinical signs included dry and orange- and/or yellow-colored feces that were dose-related based on their incidence as the dose level increased. These clinical signs were not observed during the recovery phase. They are not considered adverse based on the lack of impact on dogs health.

No RLY5016S-related effects were observed in the mean body weight, body weight change, mean food consumption values, ophthalmic, electrocardiographic, hematology, coagulation, clinical chemistry, urinalysis parameters, organ weight changes or macroscopic observations.

There were no potentially RLY5016S-related microscopic findings at the recovery sacrifice. Remaining microscopic observations in this study occurred at similar incidences and severities in control and RLY5016S-treated dogs and/or are common findings in untreated dogs of this age and breed; none are considered related to RLY5016S.

Methods

Doses:	1, 2, 3.75 g/kg/day
Frequency of dosing:	daily
Route of administration:	Oral by multiple capsules (6 maximum)
Dose volume:	na
Formulation/Vehicle:	no formulation
Species/Strain:	Dog, purebred beagle
Number/Sex/Group:	3
Age:	5 to 6 months
Weight:	7.0 to 9.7 kg for males 5.6 to 8.0 kg for females
Satellite groups:	2/sex/group Control and High Dose, for recovery
Unique study design:	no
Deviation from study protocol:	Deviations reported did not alter the study interpretation

Observations and Results

Mortality

Dogs were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress.

One control male (No. H49652) was euthanized for humane reasons on Day 273 (Week 39) due to a sudden onset of seizures that were refractory to treatment. The cause of death was brain injury that was identified microscopically as spongiosis and pallor in the cerebral cortex and hippocampus and was secondary to a hemorrhagic and neutrophilic inflammatory lesion on the mitral valve of the heart.

All other dogs survived to their scheduled sacrifice.

Clinical Signs

On dosing days, daily cageside observations were made for each dog at approximately 1 hour after each dose (based on the last animal dosed); abnormal findings were recorded. Cageside observations were discontinued on Dosing Phase Day 17. At least once during the predose phase, before dosing on Day 1, weekly thereafter, and on the day of scheduled sacrifice, detailed observations were made for each dog; abnormal findings or an indication the dog appeared normal was recorded.

RLY5016S-related clinical signs included dry and orange- and/or yellow-colored feces that were dose-related based on their incidence as the dose level increased. These clinical signs are not adverse based on the lack of impact on animal health and nonoccurrence during the recovery phase. In addition, the color of different lots of RLY5016S used in the study varied from yellow, yellow-

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orange, slightly red to red-brown in color. Since RLY5016S is not absorbed, the coloration of the feces may reflect the color of RLY5016S. Other clinical signs occurred at low incidence or frequency, and lacked a dose-related response.

Body Weights

Recorded at least once during the predose phase, on Day -1 during the predose phase, before dosing on Day 1 of the dosing phase, and weekly thereafter.

No notable effects on body weight were reported.

Feed Consumption
Quantitative food consumption was measured weekly during the dosing and recovery phases.

Significantly lower mean food consumption occurred in the 3.75 g/kg/day females during Weeks 2, 4, 14, 15, 19, and 22 with no adverse impact on mean body weight or body weight change.

Ophthalmoscopy
Performed once for all dogs during Predose Week 2 (Day 8), during Week 39 (Day 273) of the dosing phase, and during Week 4 (Day 27) of the recovery phase.

No adverse ophthalmoscopic findings were reported.

ECG
Recorded for all dogs once during Predose Week 1 (Day 7), during Week 39 (Day 273) of the dosing phase, and during Week 4 (Day 27) of the recovery phase.

No ECG abnormalities associated with treatment were reported.

Hematology

Blood samples were collected from fasted dogs for hematology, coagulation, and clinical chemistry twice during the predose phase, during Week 20 (Day 136) and during Week 40 (Day 275) of the dosing phase, and during Week 5 (Day 29) of the recovery phase.

red blood cell (erythrocyte) count	hemoglobin
hematocrit	mean corpuscular volume
mean corpuscular hemoglobin	concentration
mean corpuscular hemoglobin	platelet count
white blood cell (leukocyte) count	differential blood cell count
blood smear	reticulocyte count

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No significant changes in hematology parameters were noted in response to treatment.

Clinical Chemistry

glucose	urea nitrogen
creatinine	total protein
albumin	globulin
albumin/globulin ratio	cholesterol
triglycerides	total bilirubin
alanine aminotransferase	alkaline phosphatase
aspartate aminotransferase	calcium
inorganic phosphorus	sodium
potassium	chloride
serum protein electrophoresis	gamma glutamyltransferase

No treatment-related abnormalities in clinical chemistry parameters were noted.

Urinalysis

Urine was collected once during the predose phase, during Week 40 (Day 275) of the dosing phase, and during Week 5 (Day 29) of the recovery phase from all dogs.

appearance (clarity and color)	volume
specific gravity	ketones
bilirubin	urobilinogen
pH	protein
glucose	blood
microscopic examination of sediment	sodium
potassium	chloride
sodium excretion	potassium excretion
chloride excretion	

A mild, but significant, decrease in urine sodium excretion was observed on Day 275 of the dosing phase in males dosed at 3.75 g/kg/day. A similar but nonsignificant trend was observed in females administered the same dose. These changes resolved by Day 29 of the recovery phase and were not considered adverse.

Gross Pathology

Organ Weights

At scheduled sacrifices, the following organs (when present) were weighed; paired organs were weighed together.

adrenal (2)	brain

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epididymis (2)	heart
kidney (2)	lung
ovary (2)	prostate
salivary gland [mandibular (2)]	spleen
testis (2)	thymus
thyroid (2 lobes) with parathyroid	uterus
pituitary gland	liver with gallbladder (drained)

Histopathology

adrenal (2)	aorta	brain
cecum	cervix	colon
duodenum	epididymis (2)[a]	esophagus
eye (2)[a]	femur with bone marrow (articular surface	of the distal end)
gallbladder	heart	ileum
jejunum	kidney (2)	lacrimal gland
lesions	liver	lung with large bronchi
lymph node (mandibular)	lymph node (mesenteric)	mammary gland (females)
optic nerve (2)[a]	ovary (2)	pancreas
Peyer's patch (gut associated lymphoid	tissue)	pituitary gland
prostate	rectum	salivary gland [mandibular (2)]
sciatic nerve	skeletal muscle (thigh)	skin/subcutis
spinal cord (cervical, thoracic, and lumbar)	spleen	sternum with bone marrow
stomach	testis (2)[a]	thymus
thyroid (2 lobes) with parathyroid	tongue	trachea
urinary bladder	uterus	vagina

a Preserved in modified Davidson's fixative.

Preserved tissues listed above (as appropriate) from each animal will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

The only organ weight change of statistical significance was increased mandibular salivary gland-to-body weight ratios in the 1.0 and 3.75 g/kg/day females. Because of the lack of a dose relationship and the absence of correlating histologic findings, this is considered toxicologically insignificant. No macroscopic observations associated with RLY5016S were present.

Histological Findings

A few non-adverse microscopic findings were present only in dosing phase dogs that received RLY5016S. Dosing phase 3.75 g/kg/day female H49676 had moderate neutrophilic inflammation in the cecum that was characterized by

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infiltrates of neutrophils in the submucosa, muscularis, and mesentery. Any association with RLY5016S administration of this focal lesion is uncertain, but because of the lack of similar findings in other dogs administered RLY5016S, an association is considered unlikely.

Dosing phase 3.75 g/kg/day male H49659 had slightly increased tingible body macrophages in the Peyer's patches of the ileum and colon. This is an indication of increased lymphocyte turnover. In the absence of other gastrointestinal or lymphatic system findings, a direct association with RLY5016S is considered unlikely but uncertain.

In the pancreas, minimally to slightly increased individual cell necrosis was noted in two dosing phase 3.75 g/kg/day males. This change was characterized by an increased number of individual acinar cells with karyorrhexis or fragmentation. Because individual cell acinar necrosis is present in untreated dogs, the toxicologic significance of this increased number of necrotic cells is uncertain.

In the testis, two dosing phase 3.75 g/kg/day males and one dosing phase 2.0 g/kg/day male had slight segmental atrophy of seminiferous tubules. This is a commonly reported finding in untreated male beagles of this age and is considered unrelated to RLY5016S.

Dosing phase 3.75 g/kg/day female H49675 had slight chronic inflammation of the coronary arteries of the heart. This is another common spontaneous finding in beagles and similarly considered unrelated to RLY5016S.

There were no potentially RLY5016S-related microscopic findings at the recovery sacrifice.

Toxicokinetics

na

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7 Genetic Toxicology

An evaluation of the genotoxic potential of RLY5016 was conducted in a standard battery of *in vitro* and *in vivo* tests as summarized below. These studies were reviewed earlier under IND 75615, and copies are appended.

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

The *in vitro* bacterial reverse mutation assay was conducted in two phases, a dose range-finding study and a definitive mutagenicity assay (TR 350-07-012, titled "*Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with RLY5016"). The doses tested in the mutagenicity assay were selected based on the results from the dose range-finding study using tester strains *Salmonella typhimurium* TA100 and *Escherichia coli* WP2*uvr*A. Ten doses of RLY5016 ranging from 6.67 – 5,000 microgram/plate in the presence and absence of microsomal enzymes derived from Aroclor-induced rat liver (S9) mix were evaluated in the tester strains.

In the main study, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvr*A were used as tester strains and the RLY5016 dose levels ranged from 33.3 – 5,000 microgram/plate. The main study was also conducted in the presence and absence of S9 mix. The results of the initial mutagenicity assay were confirmed in an independent experiment. Based on the results, RLY5016 and any leachates were not mutagenic in the *in vitro* bacterial mutation assay.

7.2 *In vitro* Assays in Mammalian Cells

The ability of RLY5016 to induce chromosome aberrations was evaluated in cultured CHO cells. The study was conducted in two phases: an initial assay and a confirmatory assay (TR 350-07-013, titled "Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with RLY5016").

In the initial chromosomal aberrations assay, the treatment period was 3 hours and cultures were harvested approximately 20 hours after initiation of treatment. Concentrations of 15.6, 31.3, 62.5, 125, 250 and 500 microgram/mL were tested without and with metabolic activation. No significant increase in cells with chromosomal aberrations, polyploidy or endoreduplication was observed with or without metabolic activation in the initial assay.

Based on the results from the initial assay, the confirmatory chromosomal aberration assay was conducted at RLY5016 doses of 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL, without metabolic activation, and 31.3, 62.5, 125, 250 and 500 µg/mL with metabolic activation. Treatment periods were approximately 18 hours without metabolic activation and 3 hours with metabolic activation, and cultures were harvested approximately 20 hours after the initiation of treatment. Particulates were observed at dosing at all dose levels, at wash at all dose levels

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in cultures with metabolic activation and at dose levels ≥ 62.5 microgram/mL in cultures without metabolic activation, and at harvest at doses ≥ 125 µg/mL. No significant increase in cells with chromosomal aberrations, polyploidy or endoreduplication was observed with or without metabolic activation in the confirmatory assay.

The conclusion from this study was that RLY5016 and potential leachates were negative for inducing structural chromosomal aberrations in CHO cells with and without metabolic activation.

7.3 Clastogenicity Assay in Rodent (Micronucleus Assay)

This study (TR 350-07-014, titled "*In Vivo* Rat Bone Marrow Micronucleus Assay with RLY5016") evaluated RLY5016 for clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocytes (PCEs) in rat bone marrow. Male CD (SD)IGS BR rats were administered a single dose of RLY5016 at dosages of 0, 1, 3 and 6 g/kg (5 animals/group) via oral gavage. These dosages were chosen based on the data from the 2-week rat range-finding study (TR 350-07-009), as well as the maximum feasible concentration of the drug in 1% CMC. The frequency of micronucleated PCEs in the bone marrow of the rats was assessed at 24 hours (all dose groups) and at 48 hours (high dose group) after dosing. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 total erythrocytes for each animal.

At the 6 g/kg dose, a clear oral discharge was observed in two rats immediately post dose, but the rats appeared normal by 1-hour post dose. All remaining rats in the RLY5016-treated groups appeared normal immediately after dosing and remained healthy until the appropriate harvest time point.

RLY5016, administered at dosages up to 6 g/kg, was not cytotoxic to the bone marrow (i.e., no statistically significant decreases in the PCE:NCE ratios) and did not induce statistically significant increases in micronucleated PCEs. The vehicle control (1% CMC) group had less than approximately 0.15% micronucleated PCEs, and the group mean was within the historical control range. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs as compared to that of the vehicle control, with a mean and standard error of 3.15 ± 0.23%.

In conclusion, RLY5016 did not cause a positive increase in micronuclei in polychromatic erythrocytes in rat bone marrow.

8 Carcinogenicity

The requirement for carcinogenicity testing of RLY5016S was waived by the Agency 29 March 2012).

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Dietary Study of Fertility and Early Embryonic Development to Implantation with RL Y5016S in Rats

Study no.:	RLY-TR-0006
Study report location:	NDA 205739 SDN 0001
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	9/28/2010 (males) 10/12/2010 (females)
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	LTFLAA2002 ,55%

Key Study Findings

There were no effects on mating or the percentage of females that were impregnated, or on any cesarean section parameters, including corpora lutea, implantation sites, and fetal viability.

There were no treatment-related differences in mean absolute organ weights or organ weights as a percent of body weight.

There were no RLY5016S effects on estrous cycles or fertility parameters. The NOAEL for fertility and early embryonic development was determined to be 5 g/kg/day.

Methods

Doses:	1, 2.5 or 5 g/kg/day
Frequency of dosing:	daily
Dose volume:	na
Route of administration:	dietary
Formulation/Vehicle:	na
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	22
Satellite groups:	no
Deviation from study protocol:	The study deviations did not affect the overall interpretation of study findings or compromise the integrity of the study.

Observations and Results
At initiation of dosing, the animals were 9 weeks old and body weights ranged from 254 to 322 g for males and 182 to 233 g for females.

Mortality

Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain and distress. Once daily, cageside observations were done in conjunction with the a.m. room check.

There was no RLY5016S-related mortality.

Body Weight

All males were weighed at least once prior to treatment at randomization, on the first day of treatment prior to dosing, twice weekly during treatment, and at termination. All females were weighed at least once prior to treatment at randomization, on the first day of treatment prior to dosing, twice weekly during the premating treatment phase, and during mating. Female body weights were also recorded on GD 0, 3, 7, 10, and 13. For those females not confirmed to have mated, twice-weekly body weight measurements continued after the cessation of cohabitation.

There was a slight, dose-related decrease in male mean body weight and body weight gain at 5 g/kg/day when compared with control over the course of the study. Although the decreases did not reach statistical significance at individual intervals, they did culminate in a significant decrease in mean terminal body weight (Study Day 71) for males at 5 g/kg/day. By Study Day 71, mean male body weight at 5 g/kg/day was 7.7% less than the concurrent control weight and these males had gained 15.6% less than controls during the 70-day dosing period.

No effects on male body weight or body weight gain were noted at the 1 and 2.5 g/kg/day dose levels.

Female mean body weight and weight gain were similar to control during the premating phase with the exception of a decreased weight gain at 2.5 g/kg/day (1.7 g versus 5.4 g) during the Study Day 17 to 21 interval, which recovered by the Study Day 21 to 24 interval. This effect was considered to be spurious and unrelated to test article treatment as there were no changes in body weight or body weight gain at 5 g/kg/day in females.

There were no RLY5016S effects on mean body weight or weight gain for females during gestation.

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Feed Consumption

During the premating phase, food consumption was determined weekly for both males and females. Food consumption was not determined during mating for the males or females.

After the first week of mating, males that successfully mated were placed on measured food consumption. Food consumption was measured weekly during the post-mating phase for the males. Males that successfully mated during the second and third weeks of mating resumed measured food consumption at the beginning of the next food consumption interval. Beginning on GD 0, food consumption was measured at gestation body weight intervals for the females.

There were no test article-related effects on male food consumption.

For females, mean food consumption was significantly increased over control in the 5 g/kg/day group during the Study Day 7 to 14 and 0 to 21 intervals (premating period). During gestation, mean maternal food consumption was also significantly increased at 5 g/kg day during the GD 0 to 7 and GD 0 to 13 intervals. However, these increases did not affect mean body weight or weight gain.

Mating Procedures

Animals from respective groups were mated by placing one female in the breeding cage of a male from the same dose group. A record of mating pairs was maintained. Once mating had occurred, the males and females were separated. The maximum mating phase was 3 weeks.

During mating, a daily inspection was made for the presence of a retained copulatory plug or obvious copulatory plugs on the tray liner. Females not found with either retained or dropped copulatory plugs were evaluated for vaginal sperm by lavage. The day that sperm or plug was observed was designated GD 0.

Necropsy

Organ Weights The following tissues were weighed from males at scheduled sacrifice: epididymis (left and right), prostate, seminal vesicles (with coagulating gland), and testis (left and right).

A significant decrease in mean male terminal body weight (7.7% less than control) was observed at 5 g/kg/day when compared with control over the course of the study. There were no significant differences in mean organ weights. The significant increases in mean right and left testis weights relative to final body weight at 5 g/kg/day were attributed to the decrease in mean body weight at

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termination. There were no other significant changes in organ weights as a percent of body weight.

Male Sperm Assessment
At the scheduled sacrifice, the first 10 surviving males from each dose group were evaluated for sperm count and motility (average path velocity, straight line velocity, curvilinear velocity, straightness, and percent motility).

Sperm motility effects were limited to a significant decrease in straight-line velocity at 5 g/kg/day when compared with control (103.1 µm/s versus 110.0 µm/s), but no other treatment-related effects on sperm motility data were observed and pregnancy rates at this dose were within historical limits.

Dose Analysis

For males, week 1 diet preparations did not meet concentration specifications. At the mid and high dose, samples were 78.6% and 83% of target, respectively. Homogeneity was also outside of specifications for the high-dose preparation from week 1. Based on these low concentrations and nonhomogenous results, concentration analyses were conducted weekly from week 3 through the remainder of the study (week 10). In addition, the premating dosing period was extended by 1 week in males and females to 5 weeks and 3 weeks, respectively. This ensured that all animals were dosed for a sufficient amount of time prior to mating.

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

Estrous Cycle
There were no RLY5016S effects on estrous cycles.

Overall reproductive performance data are presented in the Table below.

	0 g/kg/day	1 g/kg/day	2.5 g/kg/day	5 g/kg/day
No. of males paired with at least one female	22	22	22	22
No. of Females mated	21	22	22	22
Coital rate (%)	95	100	100	100
No. of Females Pregnant	21	22	21	20
Conception Index (%)*	100	100	95	91
Fertility Index (%)*	95	100	95	91

* Index calculation given in Attachment No. 1 which is found after the Final Protocol

There were no treatment-related effects on the coital rate, conception, or fertility index. The fertility index was slightly decreased compared with control (91 versus 95%); however, this was well within the historical control range (84 to 100%).

Pregnancy rate was 100% for control and 1-g/kg/day dams, 95% at 2.5 g/kg/day, and 91% at 5 g/kg/day; all were within historical limits. The percent pregnant females surviving at cesarean section was 100, 95, 95, and 86, respectively.

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Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 G/KG BW/DAY	GROUP 2 1.0 G/KG BW/DAY	GROUP 3 2.5 G/KG BW/DAY	GROUP 4 G/KG BW/DAY
Females Mated	N	21	22	22	22
Pregnant	N	21	22	21	20
	%	100	100	95	91
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	21	21	21	19
Dams with Viable Fetuses	N	20	21	21	19
	%	95	100	100	100
Dams with no Viable Fetuses	N	1	0	0	0
	%	4.8	0.0	0.0	0.0
Corpora Lutea	MEAN	16.3	16.2	16.0	16.4
	S.D.	2.2	2.2	2.1	1.7
	N	21	21	21	19
	TOTAL	342	340	337	311
Implantation Sites	MEAN	15.1	15.1	15.2	15.2
	S.D.	3.3	1.6	1.5	1.3
	N	21	21	21	19
	TOTAL	318	318	320	288
Preimplantation Loss	MEAN%	7.4	5.9	4.6	6.9
	S.D.	17.0	6.7	5.4	8.6

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤ ** = P≤0.01.

9.2 Embryonic Fetal Development

Study title: Oral Gavage Study for Effects on Embryo-fetal Development with RLY5016 in Rats

Study no.:	TR 350-08-001
Study report location:	NDA 205739 SDN 0001
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	7/23/2008
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	CHZD202626

Key Study Findings

There was no RLY5016-related toxicity noted in dams or litters of dams. Therefore, the NOAEL for maternal and embryo/fetal toxicity of RLY5016 is >6000 mg/kg/day.

There were no RLY5016-related clinical observations. There were no RLY5016-related effects on mean body weight, mean body weight gain, or mean food consumption.

There were no RY5016-related necropsy findings, nor effects on gravid uterine weights, corrected weights or net change weights from day 4.

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At cesarean section, all females were confirmed to be pregnant with litters of viable fetuses. The number of corpora lutea, implantation sites, preimplantation loss, post-implantation loss, live fetuses, fetal sex ratio and mean fetal weights were not remarkable.

Methods

Doses:	1, 3 or 6 g/kg
Frequency of dosing:	daily
Dose volume:	20 mL/kg
Route of administration:	oral
Formulation/Vehicle:	suspension in water
Species/Strain:	female Crl:CD(SD) rats, timed pregnant
Number/Sex/Group:	25
Satellite groups:	no
Study design:	daily dosing GD-6 to GD-17
Deviation from study protocol:	The study deviations did not affect the overall interpretation of study findings.

Observations and Results

Mortality and Clinical Signs

Rats were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain and distress; findings were recorded as they were observed.

On dosing days, cageside observations were made approximately 1 hour postdose based on the last rat dosed in each group; abnormal findings were recorded. On non-dosing days, cageside observations were made for each rat during the a.m. room check; abnormal findings were recorded.

At each body weight interval, detailed observations were made for each rat; abnormal findings or an indication of normal was recorded.

All rats survived to the scheduled sacrifice.

There were no RLY5016-related clinical observations. Clinical observations of a sporadic nature included: swollen paw, swollen digits, damaged tip of tail, sore/scab, and alopecia.

Body Weight / Feed Consumption

Body weight was recorded on GD 4, 6, 8, 10, 12, 14, 16, 18, and 21. Beginning on GD 4, food consumption was measured at body weight intervals.
There were no RLY5016-related effects on mean body weight, mean body weight gain, and mean food consumption.

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Toxicokinetics
Not determined.

Dosing Solution Analysis

All concentration verification results met acceptance criteria (range 91.7 to 108%) with the exception of the 150 mg/mL samples at Week 2 which were 113% of target. This was not considered to be a biologically important deviation because there was no toxicity seen at this dose level. All homogeneity results met acceptance criteria (range 98.2 to 102%).

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

On GD 21, rats were sacrificed by carbon dioxide inhalation and exsanguination. Uterine contents were examined.

The fetuses were taken immediately by cesarean section and uterine contents were examined. Any grossly abnormal cervical, thoracic or abdominal viscera observations were noted. Abnormalities of the placenta or amniotic sac were described. The uterus from each gravid rat was excised, weighed, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The uterus was not reweighed after the contents were removed. The right and left ovaries from each gravid female were examined for the number of corpora lutea. For apparently nongravid females, the uterus was pressed between two glass slides and examined for implantation sites.

Offspring (Malformations, Variations, etc.)

Approximately one-half of all fetuses from each litter were processed for visceral examination. The heads were removed, frozen on dry ice, and cross-sectioned using the Wilson's sectioning technique. The internal organs of the thoracic and abdominal cavities of the fetuses were examined in the fresh state using modified Staples' technique. Viscera and carcasses were then discarded.

The remaining fetuses were eviscerated, processed for skeletal examination using the Alizarin Red S staining method, and evaluated. Findings were judged to be variations or malformations. Malformations are developmental deviations which (1) are gross structural changes, (2) are incompatible with life, or (3) may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or the well-being of the rat.

Abnormal viscera were saved in 10% neutral-buffered formalin. Fetuses processed for skeletal evaluation were retained in (b) (4)

At cesarean section, all females were confirmed to be pregnant with litters of viable fetuses. The number of corpora lutea, implantation sites, preimplantation loss, post-implantation loss, live fetuses, and fetal sex ratio were not remarkable.

There were no RLY5016-related effects on mean fetal weights.

There were no fetal external variations. Fetal external malformations were considered to be sporadic and were not considered to be related to RLY5016. Fetal soft tissue variations were considered to be sporadic and within the historical control data of this laboratory thus were not considered to be related to RLY5016. There were no soft tissue malformations.

Skeletal variations and malformation were the type commonly seen in this strain of rat and were not attributed to RLY5016.

Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	25	25	25	25
	%	100	100	100	100
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	25	25	25	25
Dams with Viable Fetuses	N	25	25	25	25
	%	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	14.0	13.8	13.8	14.1
	S.D.	1.7	2.2	2.2	2.1
	N	25	25	24	25
	TOTAL	349	344	331	352
Implantation Sites	MEAN	13.2	13.0	13.2	13.4
	S.D.	1.7	1.6	1.7	2.3
	N	25	25	25	25
	TOTAL	329	324	331	336
Preimplantation Loss	MEAN%	5.6	4.9	3.1	4.5
	S.D.	7.7	8.9	5.9	7.2

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Pregnant at C-section	N	25	25	25	25
Resorptions: Total	MEAN	0.1	0.5	0.2	0.3
	S.D.	0.3	1.0	0.5	0.6
	N	25	25	25	25
	TOTAL	3	12	5	8
	MEAN%	0.9	3.6	1.4	2.7
	S.D.	2.6	7.2	3.4	5.2
Early	MEAN	0.1	0.5	0.2	0.3
	S.D.	0.3	1.0	0.5	0.6
	N	25	25	25	25
	TOTAL	3	12	5	8
	MEAN%	0.9	3.6	1.4	2.7
	S.D.	2.6	7.2	3.4	5.2
Late	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
	N	25	25	25	25
	TOTAL	0	0	0	0
	MEAN%	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	0.9	3.6	1.4	2.7
	S.D.	2.6	7.2	3.4	5.2

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

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Summary of Mean Fetal Weights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
of all Viable Fetuses	MEAN	5.30	5.50	5.37	5.34
	S.D.	0.37	0.33	0.29	0.40
	N	25	25	25	25
Covariate Adjusted	MEAN	5.30	5.47	5.38	5.36
of Male Fetuses	MEAN	5.41	5.65	5.46	5.50
	S.D.	0.42	0.35	0.35	0.45
	N	25	25	25	25
Covariate Adjusted	MEAN	5.42	5.62	5.47	5.52
of Female Fetuses	MEAN	5.18	5.33	5.30	5.21
	S.D.	0.37	0.33	0.30	0.37
	N	25	25	25	25
Covariate Adjusted	MEAN	5.19	5.30	5.31	5.22

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Summary of Fetal External Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Litters Evaluated	N	25	25	25	25
Fetuses Evaluated	N	326	312	326	328
Live	N	326	312	326	328
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = Number

Summary of Fetal External Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Litters Evaluated	N	25	25	25	25
Fetuses Evaluated	N	326	312	326	328
Live	N	326	312	326	328
Dead	N	0	0	0	0
OPEN EYE(S)					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
APODIA					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
SYNDACTYLY					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
GASTROSCHISIS					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
BRACHYGNATHIA					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = Number

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Litters Evaluated	N	25	25	25	25
Fetuses Evaluated	N	326	312	326	328
Live	N	326	312	326	328
Dead	N	0	0	0	0
EXENCEPHALY					
Fetal Incidence	N	1	0	0	0
	%	0.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
FILAMENTOUS TAIL					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.3	0.0	0.3
Litter Incidence	N	0	1	0	1
	%	0.0	4.0	0.0	4.0
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	1	1	0	1
	%	0.3	0.3	0.0	0.3
Litter Incidence	N	1	1	0	1
	%	4.0	4.0	0.0	4.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = Number

Summary of Fetal Soft Tissue Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Litters Evaluated	N	25	25	25	25
Fetuses Evaluated	N	161	158	162	161
Live	N	161	158	162	161
Dead	N	0	0	0	0
DILATATION OF LATERAL VENTRICLE(S)					
Fetal Incidence	N	0	1	0	2
	%	0.0	0.6	0.0	1.2
Litter Incidence	N	0	1	0	2
	%	0.0	4.0	0.0	8.0
ABSENT INNOMINATE ARTERY					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.0	0.0
INCREASED RENAL PELVIC CAVITATION					
Fetal Incidence	N	2	0	0	1
	%	1.2	0.0	0.0	0.6
Litter Incidence	N	2	0	0	1
	%	8.0	0.0	0.0	4.0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	2	1	1	3
	%	1.2	0.6	0.6	1.9
Litter Incidence	N	2	1	1	3
	%	8.0	4.0	4.0	12

N = Number

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY	GROUP 4 6000 MG/KG/DAY
Litters Evaluated	N	25	25	25	25
Fetuses Evaluated	N	161	158	162	161
Live	N	161	158	162	161
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = Number

Study title: Oral Gavage Dose Range-finding Developmental Toxicity Study with RLY5016S in Rabbits

Study no.: TR 350-11-001

Study report location: NDA 205739 SDN 0001

Conducting laboratory and location: (b) (4)

Date of study initiation: 9/27/2010

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: Lot. No. LTFLAA2002, 55% purity

Key Study Findings

There were no abortions, early deliveries, dead fetuses, or any RLY5016S-related effects on the number of corpora lutea or implantation sites, mean percent resorptions, or post-implantation loss. Dose-dependent covariate-adjusted fetal weight decreases of 7.0 and 9.2% less than control were observed at 1000 and 3000 mg/kg/day RLY5016, respectively. There were no fetal external variations or malformations.

Methods

Doses: 1 or 3 g/kg (total daily dose)

Frequency of dosing: Twice daily, at least 6 hr apart

Dose volume:	5 ml/kg – low dose 10 mL/kg – high dose
Route of administration:	oral
Formulation/Vehicle:	suspension in water
Species/Strain:	female Hra:(NZW)SPF rabbits
Number/Sex/Group:	5
Satellite groups:	no
Study design:	Dosing from Gestation Day 7 through 20
Deviation from study protocol:	no protocol deviations

Observations and Results

Mortality / Clinical Signs

Rabbits were observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain and distress. Once daily, cageside observations were performed on all rabbits in conjunction with the a.m. room check. In addition, postdose observations were made for each rabbit on GD 7 through 20 at approximately 1 hour postdose (after each dose) based on the last rabbit dosed per group. At each body weight interval, detailed observations were made for each rabbit.

All rabbits survived to scheduled necropsy.

There were no RLY5016S-related clinical observations. Sporadic findings of few or no feces were noted in one control and one 3000 mg/kg/day rabbit.

Body Weight

Recorded prior to dosing and on GD 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27, and 29.

Mean maternal body weights were similar to control for both treated groups. Mean weight change data were highly variable during the study. After dosing began on GD 7, mean weight gain at 1000 and 3000 mg/kg/day was generally higher than control with an overall weight gain of 147.6, 185.8, and 224.2 g, for the three groups over the GD 7 to 21 interval. The increase in mean body weight gain over control observed in the treated groups can largely be attributed to low body weight and food consumption noted for a single Control Dam (No. F72570).

Feed Consumption

Consumption was measured quantitatively daily beginning on GD 4.

Mean food consumption for rabbits dosed at 1000 and 3000 mg/kg/day were generally higher than control: Dam No. F72570, discussed above, was primarily responsible for the low overall mean food consumption noted in the control group

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from GD 9 through 17. This rabbit ate 86 g of food for the GD 9 to10 interval with food consumption of 1 or 2 g from GD 11 through 15. Vegetable supplements were given to this rabbit over these intervals.

After GD 18, mean food consumption was generally 10 to 12% less than control at 1000 mg/kg/day and was generally 5 to 9% less than control at 3000 mg/kg/day.

Toxicokinetics

Not determined.

Dosing Solution Analysis

All formulations were considered acceptable for use under the conditions of this study. Results were within 10% of target concentrations.

Necropsy

There were no macroscopic findings in dams at necropsy.

Cesarean Section Data

There was a nonpregnant rabbit in each treated group, resulting in a pregnancy rate of 80% for both groups (pregnancy rate was 100% for control, however, since implantation occurs prior to GD 7, the reduction in pregnancy rate is not attributed to RLY5016S.

There were no abortions, early deliveries, or dead fetuses and no RLY5016S-related effects on the number of corpora lutea, implantation sites, number or mean percent resorptions, or post-implantation loss.

Fetal Weights
Dose-dependent fetal weight decreases of 7.0 and 9.2% less than control were observed at 1000 and 3000 mg/kg/day, respectively.

Offspring (Malformations, Variations, etc.)

Fetal External Evaluations

There were no fetal external variations or malformations

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Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY
Females Mated	N	5	5	5
Pregnant	N	5	4	4
	%	100	80	80
Aborted	N	0	0	0
	%	0.0	0.0	0.0
Died	N	0	0	0
	%	0.0	0.0	0.0
Delivered Early	N	0	0	0
	%	0.0	0.0	0.0
Pregnant at C-section	N	5	4	4
Dams with Viable Fetuses	N	5	4	4
	%	100	100	100
Dams with no Viable Fetuses	N	0	0	0
	%	0.0	0.0	0.0
Corpora Lutea	MEAN	9.4	10.2	9.5
	S.D.	1.9	1.3	1.0
	N	5	4	4
	TOTAL	47	41	38
Implantation Sites	MEAN	8.0	9.5	9.0
	S.D.	2.0	1.7	0.8
	N	5	4	4
	TOTAL	40	38	36
Preimplantation Loss	MEAN%	12.9	7.5	5.1
	S.D.	21.7	9.6	5.9

Non-pregnant rabbits excluded from analysis

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY
Pregnant at C-section	N	5	4	4
Resorptions: Total	MEAN	0.6	0.0	0.2
	S.D.	0.5	0.0	0.5
	N	5	4	4
	TOTAL	3	0	1
	MEAN%	6.5	0.0	3.1
	S.D.	6.0	0.0	6.2
Early	MEAN	0.6	0.0	0.2
	S.D.	0.5	0.0	0.5
	N	5	4	4
	TOTAL	3	0	1
	MEAN%	6.5	0.0	3.1
	S.D.	6.0	0.0	6.2
Late	MEAN	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0
	N	5	4	4
	TOTAL	0	0	0
	MEAN%	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0
Dead Fetuses	TOTAL	0	0	0
Postimplantation Loss	MEAN%	6.5	0.0	3.1
	S.D.	6.0	0.0	6.2

Non-pregnant rabbits excluded from analysis

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY
Pregnant at C-section	N	5	4	4
Live Fetuses	MEAN	7.4	9.5	8.8
	S.D.	1.5	1.7	1.3
	N	5	4	4
	TOTAL	37	38	35
	MEAN%	93.5	100.0	96.9
	S.D.	6.0	0.0	6.2

Summary of Mean Fetal Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 1000 MG/KG/DAY	GROUP 3 3000 MG/KG/DAY
FETAL WEIGHTS UNITS: GRAMS of all Viable Fetuses	MEAN	40.58	36.33	35.95
	S.D.	2.60	1.50	4.89
	N	5	4	4
Covariate Adjusted MEAN		39.83	37.06	36.15

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05
** = P≤0.01.

9.3 Prenatal and Postnatal Development

Prenatal and postnatal reproductive toxicology studies were not conducted.

10 Special Toxicology Studies

No special toxicology studies were conducted.

Reference ID: 3781906

11 Appendix/Attachments

From review of IND 75,615 SDN 000

Genetic Toxicology Studies

Study title: *Salmonella-Escherichia coli*/Mammalian- Microsome Reverse Mutation Assay with a Confirmatory Assay with ILY105

Key findings: No positive increase in the mean number of revertants per plate with any of the tester strains were detected, either in the presence or absence of S9 metabolic activation

Study no.: TR 350-07-012; 27616-0-4090ECD; (b) (4) Study No. 7578-120
Volume #, and page #: *Vol. 5.12: pg 0*01
Conducting laboratory and location: (b) (4)

Date of study initiation: 5/23/06
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: ILY105 Batch No. CHTLO0261

Methods

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2uvrA. The specific genotypes of the strains are shown in Table I.

Table I. Tester Strain Genotypes

Tester Strain	*his*/*trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A	*trp*	*uvr*A	–	–

The combinations of positive controls, activation conditions, and tester strains plated concurrently with the assay are indicated in Table II.

Table II. Positive Controls			
Tester Strain	S9 Mix	Positive Control	Dose (μg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Liver microsomal enzymes (S9 homogenate) were purchased from (b) (4) , Lot No. 2001 (40.5 mg protein/mL). The homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 (200 mg/mL in corn oil) at 500 mg/kg. The S9 mix was prepared immediately prior to its use in any experimental procedure.

Dose Rangefinding Study: The growth inhibitory effect (cytotoxicity) of the test article to the test system was determined in order to allow the selection of appropriate doses to be tested in the mutagenicity assay. The dose rangefinding study was performed using tester strains TA100 and WP2uvrA in both the presence and absence of S9 mix. Ten doses of ILY105 were tested at one plate per dose and checked for cytotoxicity up to a maximum concentration of 5000 μg/plate. Since no cytotoxicity was observed in the dose rangefinding study, the highest dose level of test article used in the mutagenicity assay was the same dose as that tested in the rangefinding study.

Results

Dose Rangefinding Study
Doses tested in the mutagenicity assay were selected based on the results of the dose rangefinding assay conducted on the test article using tester strains TA100 and WP2uvrA in both the presence and absence of S9 mix with one plate per dose. Ten doses of ILY105 ranging from 6.67 to 5000 μg/plate were tested and results are presented in Table 1. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix.

Mutagenicity Assay
Mutagenicity results for ILY105 are presented in Tables 2 through 6. These data were generated in Trials 27616-B1, 27616-C1, and 27616-01. Data are presented as individual plate counts (Tables 2 and 4, not shown) and as mean revertants per plate ± standard deviation (Tables 3, 5, and 6) for each treatment and control group.

Results of the dose rangefinding study were used to select doses tested in the mutagenicity assay. Doses tested in the mutagenicity assay with all tester strains

in both the presence and absence of S9 mix were 33.3, 100, 333, 1000, 3330, and 5000 μg/plate.

In the initial mutagenicity assay, Trial 27616-B1 (Table 3), the mean positive control value for TA100 in the presence of S9 mix did not exhibit at least a 3-fold increase over the mean vehicle control value. In addition, the mean positive control values for TA 1535 and TA1537 in the presence of S9 mix were lower than routinely observed (although they did exhibit >3-fold increases over the appropriate mean vehicle control values). For these reasons, the test article was re-tested in the presence of S9 mix with tester strains TA100, TA 1535, and TA1537 in Trial 27616-01. All other data generated in Trial 27616-81 were acceptable and no positive increases in the mean number of revertants per plate were observed with any of the remaining tester strain/activation condition combinations.

In the confirmatory mutagenicity assay, Trial 27616-C1 (Table 5), all data were acceptable and no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the repeat mutagenicity assay, Trial 27616-01 (Table 6), all data were acceptable and no positive increases in the mean number of revertants per plate were observed with tester strains TA100, TA1535, or TA1537 in the presence of S9 mix.

Conclusions

ILY105 tested in the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of S9 metabolic activation.

Reference ID: 3781906

Table 1 : Dose Rangefinding Study

Test Article ID: ILY105

Assay No.: 27616-0-409OECD | Trial No.: A1

Date Plated: 13-Jun-06 | Vehicle: Saline

Date Counted: 16-Jun-06 | Plating Aliquot: 1000 µL

		Revertants per Plate			
	Dose/Plate	TA100	Background Lawn[a]	WP2*uvr*A	Background Lawn[a]
Microsomes: Rat Liver					
Vehicle Control		122	N	10	N
Test Article	6.67 µg	115	N	19	N
	10.0 µg	91	N	12	N
	33.3 µg	93	N	14	N
	66.7 µg	97	N	19	NP
	100 µg	124	N	16	NP
	333 µg	114	N	14	NP
	667 µg	103	NP	8	NP
	1000 µg	114	NP	17	NP
	3330 µg	86	NP	10	NP
	5000 µg	108	NP	9	NP
Microsomes: None					
Vehicle Control		38	N	15	N
Test Article	6.67 µg	84	N	18	N
	10.0 µg	85	N	16	N
	33.3 µg	61	N	11	N
	66.7 µg	91	N	17	N
	100 µg	77	N	21	NP
	333 µg	95	N	15	NP
	667 µg	85	NP	10	NP
	1000 µg	53	NP	17	NP
	3330 µg	87	NP	10	NP
	5000 µg	60	NP	16	NP

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

Table 3 : Mutagenicity Assay Results – Summary

Test Article ID: ILY105

Assay No.: 27616-0-409OECD Trial No.: B1

Date Plated: 29-Jun-06 Vehicle: Saline

Date Counted: 07-Jul-06 Plating Aliquot: 1000 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		16	2	99	2	11	5	6	2	19	2	N
Test Article	33.3 µg	17	4	90	6	11	2	7	1	16	2	N
	100 µg	20	6	96	12	9	3	5	5	19	6	N
	333 µg	16	3	89	11	11	1	10	2	16	5	NP
	1000 µg	28	6	94	11	10	1	7	2	20	3	NP
	3330 µg	22	7	91	10	10	4	5	2	21	5	NP
	5000 µg	20	5	98	5	12	4	4	3	21	6	NP
Positive Control[b]		170	11	258	38	54	9	67	48	366	25	N
Microsomes: None												
Vehicle Control		15	0	90	5	10	5	4	3	11	6	N
Test Article	33.3 µg	11	5	88	3	12	7	6	3	12	5	N
	100 µg	12	6	90	9	15	4	4	2	13	4	N
	333 µg	10	2	81	11	6	1	6	4	15	5	NP
	1000 µg	13	2	76	16	8	3	4	3	18	4	NP
	3330 µg	8	3	87	16	8	1	6	4	7	2	NP
	5000 µg	14	8	90	4	9	3	6	3	14	9	NP
Positive Control[c]		148	25	1125	120	752	117	205	57	132	6	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]

TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]

TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Table 5 : Mutagenicity Assay Results – Summary

Test Article ID: ILY105

Assay No.: 27616-0-409OECD Trial No.: C1

Date Plated: 14-Jul-06 Vehicle: Saline

Date Counted: 19-Jul-06 Plating Aliquot: 1000 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		15	4	97	11	14	10	4	2	20	6	N
Test Article	33.3 µg	11	5	93	4	9	3	7	2	13	3	N
	100 µg	15	3	115	16	10	1	7	3	16	6	N
	333 µg	17	3	115	3	13	5	10	4	15	4	NP
	1000 µg	19	3	106	5	7	2	5	2	15	3	NP
	3330 µg	11	2	106	8	10	2	3	2	19	5	NP
	5000 µg	12	8	113	6	8	2	2	1	21	3	NP
Positive Control[b]		454	11	1035	267	149	19	117	10	302	77	N
Microsomes: None												
Vehicle Control		11	4	89	11	10	2	6	3	16	5	N
Test Article	33.3 µg	14	2	85	9	9	1	7	2	17	5	N
	100 µg	13	2	90	12	10	6	5	3	20	2	N
	333 µg	14	3	101	16	9	2	8	1	17	2	NP
	1000 µg	13	7	83	7	9	3	7	5	16	2	NP
	3330 µg	11	2	90	8	9	1	5	2	13	4	NP
	5000 µg	11	1	97	16	11	2	3	2	17	1	NP
Positive Control[c]		316	23	1255	130	870	33	312	35	182	10	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]

TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]

TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Table 6 : Mutagenicity Assay Results – Individual Plate Counts and Summary

Test Article ID: ILY105

Assay No.: 27616-0-409OECD Trial No.: D1

Date Plated: 04-Aug-06 Vehicle: Saline

Date Counted: 07-Aug-06 Plating Aliquot: 1000 µL

Revertants Per Plate

	Dose/Plate	TA100			TA1535			TA1537			Background Lawn[a]
		1	2	3	1	2	3	1	2	3	
Microsomes: Rat Liver											
Vehicle Control		91	101	114	10	11	15	2	9	10	N
Test Article	33.3 µg	122	123	104	14	13	15	3	8	10	N
	100 µg	114	102	91	10	8	12	8	3	10	NP
	333 µg	108	111	124	10	10	14	1	5	5	NP
	1000 µg	116	122	112	15	9	12	12	4	6	NP
	3330 µg	89	120	96	13	11	14	7	11	12	NP
	5000 µg	125	133	116	8	12	11	8	8	4	NP
Positive Control[b]		723	689	542	122	113	126	101	123	108	N

Mean Revertants Per Plate with Standard Deviation

	Dose/Plate	TA100		TA1535		TA1537		Background Lawn[a]
		Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver								
Vehicle Control		102	12	12	3	7	4	N
Test Article	33.3 µg	116	11	14	1	7	4	N
	100 µg	102	12	10	2	7	4	NP
	333 µg	114	9	11	2	4	2	NP
	1000 µg	117	5	12	3	7	4	NP
	3330 µg	102	16	13	2	10	3	NP
	5000 µg	125	9	10	2	7	2	NP
Positive Control[b]		651	96	120	7	111	11	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate

Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with ILY105

Key findings: Negative for inducing chromosomal aberrations, polyploidy, and endoreduplication with or without metabolic activation.

Study no.: TR 350-07-013; 27616-0-4370ECD; (b) (4) Study Number 7578-121

Volume #, and page #: *Vol. 5.12; pg. 54*
Conducting laboratory and location: (b) (4)

Date of study initiation: 6/1/06
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: ILY105, Batch No. CHTL00261

Methods

Test System: Chinese hamster ovary cells (CHO-WBL) were used in this assay. (b) (4)

Controls

Negative and Vehicle Controls: In the nonactivation assays, negative controls were cultures, which contained only cells and culture medium. Vehicle controls were cultures containing saline at 100 μL/mL, the highest concentration used in test cultures. In the activation assays, the negative and vehicle controls were the same as described in the nonactivation assays but with the S9 activation mix included.

Positive Control Agents: The positive control agents which were used in the chromosomal aberrations assays were mitomycin C (MMC) for the nonactivation series and cyclophosphamide (CP) in the metabolic activation series. Two concentrations of MMC (0.750 and 1.50 μg/mL, for the 3-hour treatment, 0.200 and 0.400 μg/mL, for approximately 18-hour treatment) and CP (7.50 and 12.5 μg/mL) were used to induce chromosomal aberrations in the CHO cells. One of the concentrations was analyzed in each of the aberration assays. Both MMC and CP were dissolved in water.

Reference ID: 3781906

S9 Metabolic Activation System: The metabolic activation system consisted of a rat liver post-mitochondrial fraction (S9) after treatment of the rats with Aroclor™ 1254 (single concentration of 500 mg/kg) and sacrificed 5 days later.

In the chromosomal aberrations assay, replicate cultures were used at each concentration, the negative and vehicle controls, and for each of two concentrations of the positive control articles. A single approximately 20-hour harvest time without and with S9 was conducted. This harvest time corresponds to 1.5 times a cell cycle time of approximately 13 hours.

In the confirmatory chromosomal aberrations assays, replicate cultures were used at each concentration, the negative and vehicle controls, and for each of two concentrations of the positive control articles. In the confirmatory aberrations assay without and with S9, approximately 20-hour harvests were conducted.

One hundred cells, if possible, from each replicate culture from four concentrations of the test article, the vehicle, negative, and one dose of the positive control cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962, 1976). At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations. Mitotic index was evaluated from the negative and vehicle control, and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as a percentage of mitotic cells. Percent polyploidy and endoreduplication were also analyzed by evaluating at least 100 metaphases, if available, and tabulated. For control of bias, all slides were coded prior to analysis.

Results

Initial Chromosomal Aberrations Assay

In the assay without metabolic activation, a particulate was observed at dosing and at wash at all dose levels, and at harvest at 500 μg/mL. Chromosomal aberrations were analyzed from the cultures treated with 62.5, 125, 250, and 500 μg/mL (Table 3). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

In the assay with metabolic activation, a particulate was observed at dosing and at wash at all dose levels, and at harvest at ≥250 μg/mL. Chromosomal aberrations were analyzed from the cultures treated with 62.5, 125, 250, and 500 μg/mL (Table 6). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Reference ID: 3781906

Confirmatory Chromosomal Aberrations Assay

Based on the results from the initial assay, the confirmatory chromosomal aberrations assay was conducted at doses of 15.6, 31.3, 62.5, 125, 250, and 500 μg/mL without metabolic activation and 31.3, 62.5, 125, 250, and 500 μg/mL with metabolic activation. Treatment periods were approximately 18 hours without metabolic activation and 3 hours with metabolic activation. Cultures were harvested approximately 20 hours from the initiation of treatment.

In the assay without metabolic activation, a particulate was observed at dosing at all dose levels, at wash at ≥ 62.5 μg/mL, and at harvest at ≥ 125 μg/mL. Chromosomal aberrations were analyzed from the cultures treated with 62.5, 125, 250, and 500 μg/mL (Table 9). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

In the assay with metabolic activation, a particulate was observed at dosing and at wash at all dose levels, and at harvest at ≥ 125 μg/mL. Chromosomal aberrations were analyzed from the cultures treated with 62.5, 125,250, and 500 μg/mL (Table 12). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Under nonactivation conditions, the sensitivity of the cell cultures for induction of chromosomal aberrations is shown by the increased frequency of aberrations in the cells exposed to mitomycin C, the positive control agent. The test article, ILY1 05, was considered negative for inducing chromosomal aberrations, polyploidy, and endoreduplication without metabolic activation.

The successful activation by the metabolic system is illustrated by the increased incidence of cells with chromosomal aberrations in the cultures induced with cyclophosphamide, the positive control agent. The test article, ILY105, was considered negative for inducing chromosomal aberrations, polyploidy, and endoreduplication with metabolic activation.

Conclusions

ILY105 was considered negative for inducing structural chromosomal aberrations in CHO cells without and with metabolic activation.

Reference ID: 3781906

Table 3: Chromosomal Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation - 3-Hour Treatment, ~20-Hour Harvest

Assay No.: 27616-0-437OECD Trial No.: B1 Date: 06/01/06 Lab No.: CY052906A Test Article: ILY105

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	4	0			2		1		2	2	
			B	100		100	3	0		1					0	1	
			Total	**200**		**200**				1	2		1		2	3	
			Average	%	--		**3.5**	**0.0**		**0.5**	**1.0**		**0.5**		**1.0**	**1.5**	
Vehicle:	0.9% saline	100 µL/mL	A	100		100	2	0							0	0	
			B	100		100	2	0					1		1	1	
			Total	**200**		**200**							1		1	1	
			Average	%	**0**		**2.0**	**0.0**					**0.5**		**0.5**	**0.5**	
Positive:	MMC	0.750 µg/mL	A	50		100	5	0		1	7	10	1	1	16	16	
			B	50		100	3	0		1	5	16		1	21	21	
			Total	**100**		**200**				2	12	26	1	2	37	37	
			Average	%	--		**4.0**	**0.0**	-	**2.0**	**12.0**	**26.0**	**1.0**	**2.0**	**37.0**	**37.0**	+
Test Article		62.5 µg/mL	A	100		100	1	0		1					0	1	
			B	100		100	2	0					1		1	1	
			Total	**200**		**200**				1			1		1	2	
			Average	%	**0**		**1.5**	**0.0**	-	**0.5**			**0.5**		**0.5**	**1.0**	-
		125 µg/mL	A	100		100	6	0		2					0	2	
			B	100		100	3	0		1			2		2	3	
			Total	**200**		**200**				3			2		2	5	
			Average	%	**12**		**4.5**	**0.0**	-	**1.5**			**1.0**		**1.0**	**2.5**	-
		250 µg/mL	A	100		100	2	0		2					0	2	
			B	100		100	2	0		1					0	1	
			Total	**200**		**200**				3					0	3	
			Average	%	**13**		**2.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		500 µg/mL	A	100		100	2	0							0	0	
			B	100		100	3	0		2		1			1	3	
			Total	**200**		**200**				2		1			1	3	
			Average	%	**15**		**2.5**	**0.0**	-	**1.0**		**0.5**			**0.5**	**1.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium MMC = Mitomycin C

Reference ID: 3781906

Table 6: Chromosomal Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation - 3-Hour Treatment, ~20-Hour Harvest

Assay No.: 27616-0-437OECD Trial No.: B1 Date: 06/01/06 Lab No.: CY052906A Test Article: ILY105

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	6	0		1					0	1	
			B	100		100	5	0		2					0	2	
			Total	**200**		**200**				3					0	3	
			Average	%	--		**5.5**	**0.0**		**1.5**					**0.0**	**1.5**	
Vehicle:	0.9% saline	100 µL/mL	A	100		100	3	0		2					0	2	
			B	100		100	7	0		1			1		1	2	
			Total	**200**		**200**				3			1		1	4	
			Average	%	**0**		**5.0**	**0.0**		**1.5**			**0.5**		**0.5**	**2.0**	
Positive:	CP	12.5 µg/mL	A	31		100	6	2		4	12	10	1		14	14	
			B	33		100	4	0			4	7	1		11	11	
			Total	**64**		**200**				4	16	17	2		25	25	
			Average	%	--		**5.0**	**1.0**	-	**6.3**	**25.0**	**26.6**	**3.1**		**39.1**	**39.1**	+
Test Article		62.5 µg/mL	A	100		100	6	2		1					0	1	
			B	100		100	3	0		2					0	2	
			Total	**200**		**200**				3					0	3	
			Average	%	**0**		**4.5**	**1.0**	-	**1.5**					**0.0**	**1.5**	-
		125 µg/mL	A	100		100	6	0							0	0	
			B	100		100	4	1		1					0	1	
			Total	**200**		**200**				1					0	1	
			Average	%	**0**		**5.0**	**0.5**	-	**0.5**					**0.0**	**0.5**	-
		250 µg/mL	A	100		100	3	0		2					0	2	
			B	100		100	4	0		1			1		1	2	
			Total	**200**		**200**				3			1		1	4	
			Average	%	**0**		**3.5**	**0.0**	-	**1.5**			**0.5**		**0.5**	**2.0**	-
		500 µg/mL	A	100		100	8	0			1		1		2	2	
			B	100		100	4	0		1					0	1	
			Total	**200**		**200**				1	1		1		2	3	
			Average	%	**0**		**6.0**	**0.0**	-	**0.5**	**0.5**		**0.5**		**1.0**	**1.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium CP = Cyclophosphamide

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Table 9: Chromosomal Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation - ~18-Hour Treatment, ~20-Hour Harvest

Assay No.: 27616-0-437OECD Trial No.: C1 Date: 06/27/06 Lab No.: CY062606A Test Article: ILY105

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple				Totals[c]		
										gaps	breaks	chte	chre	mab	-g	+g	
Controls																	
Negative:	McCoy's 5a		A	100		100	4	0		2			2		2	4	
			B	100		100	6	0		5			1		1	5	
			Total	**200**		**200**				7			3		3	9	
			Average	%	--		**5.0**	**0.0**		**3.5**			**1.5**		**1.5**	**4.5**	
Vehicle:	0.9% saline	100 µL/mL	A	100		100	5	0		2					0	2	
			B	100		100	5	0			2		3		4	4	
			Total	**200**		**200**				2	2		3		4	6	
			Average	%	**0**		**5.0**	**0.0**		**1.0**	**1.0**		**1.5**		**2.0**	**3.0**	
Positive:	MMC	0.200 µg/mL	A	75		100	1	0		22	9	15	1		21	37	
			B	50		100	4	0		7	5	12	1		16	20	
			Total	**125**		**200**				29	14	27	2		37	57	
			Average	%	--		**2.5**	**0.0**	-	**23.2**	**11.2**	**21.6**	**1.6**		**29.6**	**45.6**	+
Test Article		62.5 µg/mL	A	100		100	2	0		3					0	3	
			B	100		100	3	0		4			1		1	5	
			Total	**200**		**200**				7			1		1	8	
			Average	%	**11**		**2.5**	**0.0**	-	**3.5**			**0.5**		**0.5**	**4.0**	-
		125 µg/mL	A	100		100	5	0		2			3		3	5	
			B	100		100	5	0		3			5		5	8	
			Total	**200**		**200**				5			8		8	13	
			Average	%	**0**		**5.0**	**0.0**	-	**2.5**			**4.0**		**4.0**	**6.5**	-
		250 µg/mL	A	100		100	1	1		2			4		4	6	
			B	100		100	3	0		4			3		3	7	
			Total	**200**		**200**				6			7		7	13	
			Average	%	**0**		**2.0**	**0.5**	-	**3.0**			**3.5**		**3.5**	**6.5**	-
		500 µg/mL	A	100		100	3	0		2					0	2	
			B	100		100	2	0		3			1		1	4	
			Total	**200**		**200**				5			1		1	6	
			Average	%	**0**		**2.5**	**0.0**	-	**2.5**			**0.5**		**0.5**	**3.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium MMC = Mitomycin C

Reference ID: 3781906

Table 12: Chromosomal Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation - 3-Hour Treatment, ~20-Hour Harvest

Assay No.: 27616-0-437OECD Trial No.: C1 Date: 06/27/06 Lab No.: CY062606A Test Article: ILY105

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	3	0		2	1		3		4	6	
			B	100		100	5	0		5			5		5	10	
			Total	**200**		**200**				7	1		8		9	16	
			Average	%	--		**4.0**	**0.0**		**3.5**	**0.5**		**4.0**		**4.5**	**8.0**	
Vehicle:	0.9% saline	100 µL/mL	A	100		100	2	0		1			1		1	2	
			B	100		100	3	0		3					0	3	
			Total	**200**		**200**				4			1		1	5	
			Average	%	**0**		**2.5**	**0.0**		**2.0**			**0.5**		**0.5**	**2.5**	
Positive:	CP	12.5 µg/mL	A	50		100	5	0		10	8	7		11	22	28	
			B	50		100	6	0		13	12	11	1	9	28	37	
			Total	**100**		**200**				23	20	18	1	20	50	65	
			Average	%	--		**5.5**	**0.0**	-	**23.0**	**20.0**	**18.0**	**1.0**	**20.0**	**50.0**	**65.0**	+
Test Article		62.5 µg/mL	A	100		100	4	0		2	1	1	2		4	5	
			B	100		100	3	0		2			3		3	5	
			Total	**200**		**200**				4	1	1	5		7	10	
			Average	%	**0**		**3.5**	**0.0**	-	**2.0**	**0.5**	**0.5**	**2.5**		**3.5**	**5.0**	-
		125 µg/mL	A	100		100	5	1		3			2		2	5	
			B	100		100	4	1		1			1		1	2	
			Total	**200**		**200**				4			3		3	7	
			Average	%	**2**		**4.5**	**1.0**	-	**2.0**			**1.5**		**1.5**	**3.5**	-
		250 µg/mL	A	100		100	5	1							0	0	
			B	100		100	6	0		3			4		4	6	
			Total	**200**		**200**				3			4		4	6	
			Average	%	**0**		**5.5**	**0.5**	-	**1.5**			**2.0**		**2.0**	**3.0**	-
		500 µg/mL	A	100		100	5	1		1			1		1	2	
			B	100		100	9	3		4			1		1	5	
			Total	**200**		**200**				5			2		2	7	
			Average	%	**4**		**7.0**	**2.0**	-	**2.5**			**1.0**		**1.0**	**3.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control. [b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium CP = Cyclophosphamide

Study title: *In Vivo* Rat Bone Marrow Micronucleus Assay with ILY105

Key findings: ILY105 did not cause a positive increase in micronuclei in polychromatic erythrocytes in rat bone marrow at single oral doses up to 6000 mg/kg.

Study no.: TR 350-07-014: 27616-0-4540ECD; (b) (4) Study Number 7578-122
Volume #, and page #: *Vol. 5.12: pg. 114*
Conducting laboratory and location: (b) (4)

Date of study initiation: 5/30/06
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: CHTL00261

Methods

Young adult male CD (SD)IGS BR rats were used. Animals were acclimated to laboratory conditions for at least 5 days, and released for study use by a staff veterinarian.
Based on the data from a 2-week range-finding study ((b) (4) 7578-116), as well as the determination of the maximum feasible concentration of ILY105 in 1% CMC, the doses selected for the micronucleus assay were 1000, 3000, and 6000 mg/kg. At initiation of treatment, the rats were approximately 8 weeks old, and their body weights ranged from 255 to 297g.

Rats were assigned to study groups as follows.

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest Timepoint 24 Hour Male	48 Hour Male
Positive Control, 60	6	10	Oral Gavage	5	-
Vehicle Control, 0	0	20	Oral Gavage	5	5
1000	50	20	Oral Gavage	5	-
3000	150	20	Oral Gavage	5	-
6000	300	20	Oral Gavage	5	5

Vehicle Control = 1% aqueous carboxymethyl cellulose solution, Positive Control = Cyclophosphamide

The treatment regimen was single oral gavage dose administration. The oral route was selected because it is the intended route of administration in humans. Prior to dosing, the top stock of ILY105 was prepared by adding the appropriate volume of the vehicle, 1.0% aqueous carboxymethyl cellulose solution, to a pre-weighed quantity of ILY105 and mixed, forming a homogeneous suspension. Lower concentrations were obtained by dilution with the vehicle.

All surviving rats were euthanized by CO_2 inhalation followed by incision of the diaphragm, and discarded without necropsy.

Extraction of Bone Marrow: The hind limb bones (tibias) were removed for marrow extraction from all surviving rats in each treatment and control group. For each rat, the

Reference ID: 3781906

marrow flushed from the bones was combined in an individual centrifuge tube containing 3 to 5 mL fetal bovine serum (one tube per animal).

Preparation of Slides: Following centrifugation to pellet the marrow, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air-dried. The slides were fixed in methanol, stained in acridine orange, and analyzed under fluorescent microscopy. For control of bias, all slides were coded prior to analysis.

Slides prepared from the bone marrow collected from all surviving rats per group at the designated harvest timepoints were scored for micronuclei and the PCE to NCE cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCEs from at least 2000 PCEs per animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal.

The 1000, 3000, and 6000 mg/kg dose groups, as well as the positive control group, were compared with the vehicle control group at the 5%, one-tailed probability level.

Results

Two rats in the 6000 mg/kg dose group were observed with a clear oral discharge immediately post dose, but appeared normal by the 1-hour post dose observation interval. All remaining rats in the ILY105-treated groups appeared normal immediately after dosing and remained healthy until the appropriate harvest timepoint. All rats in the vehicle and positive control groups appeared normal after dosing and remained healthy until the appropriate harvest timepoint.

ILY105, administered at doses up to 6000 mg/kg was not cytotoxic to the bone marrow (i.e., no statistically significant decreases in the PCE:NCE ratios), and did not induce statistically significant increases in micronucleated PCEs.

The vehicle control group had less than approximately 0.15% micronucleated PCEs and the group mean was within the historical control range. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs as compared to that of the vehicle control, with a mean and standard error of 3.15 ± 0.23%.

Conclusion

Male rats were administered a single dose of ILY105 by oral gavage at 0, 1000, 3000, and 6000 mg/kg. ILY105 did not cause a positive increase in micronuclei in polychromatic erythrocytes in rat bone marrow.

Table 1: Micronucleus Assay – Summary Table

Assay No.: 27616-0-454OECD
Test Article: ILY105
Initiation of Dosing: 30 May 2006

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	1% CMC 20 mL/kg	24 hr	0.06 ± 0.03	0.88 ± 0.09
		48 hr	0.06 ± 0.01	0.97 ± 0.07
Positive	CP 60 mg/kg	24 hr	3.15 ± 0.23*	0.75 ± 0.06
Test Article	1000 mg/kg	24 hr	0.08 ± 0.03	0.80 ± 0.05
	3000 mg/kg	24 hr	0.08 ± 0.05	0.96 ± 0.08
	6000 mg/kg	24 hr	0.02 ± 0.01	0.79 ± 0.07
		48 hr	0.07 ± 0.03	0.82 ± 0.03

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
CMC = Carboxymethylcellulose
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

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/s/

WILLIAM T LINK
06/19/2015

ALBERT F DEFELICE
06/19/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 205739 **Applicant: Relypsa, Inc.** **Stamp Date:**

Drug Name: **NDA/BLA Type:**

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?	yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	yes		
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)?	yes		
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).	no		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	yes		
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?	yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	yes		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?			A direct mg/kg comparison (animal/man) is appropriate as the drug product is not absorbed.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	yes		
11	Has the applicant addressed any abuse potential issues in the submission?			Abuse potential is highly unlikely
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/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

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12/03/2014

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12/03/2014