CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
22-554

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22,554
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Applicant's letter date:	June 24, 2009
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Product:	XIFAXAN® (rifaximin)
Indication:	Hepatic Encephalopathy
Applicant:	Salix Pharmaceuticals, Inc.
Review Division:	Division of Gastroenterology Products
Reviewer:	Niraj R. Mehta, Ph.D.
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Division Director:	Donna Griebel, M.D.
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TABLE OF CONTENTS

Table of Tables

1 Executive Summary

1.1 Recommendations

The recommendations are presented below in sections 1.1.1, 1.1.2 and 1.1.3.

1.1.1 Approvability

The present application for Xifaxan (rifaximin) should be approved. The proposed indication is maintenance of remission of hepatic encephalopathy. I defer to the other review team members for evaluation of the appropriateness of this indication. The Sponsor must conduct the post–marketing nonclinical studies to address the safety concern about the high plasma exposure in cirrhotic patients, as detailed in Section 1.1.2.

1.1.2 Additional Non Clinical Recommendations

The incidence of hepatotoxicity in nonclinical studies, albeit inconsistent, raises a safety concern for the intended patient population (i.e., cirrhosis patients with hepatic encephalopathy). This concern is compounded by the results of clinical study RFHE3002PK, which showed an increase in plasma drug levels in cirrhotic patients. At the proposed dose of 550 mg bid, the AUC in cirrhotic patients was 10 to 20-fold higher than that observed in healthy volunteers. The increase in AUC was correlated with severity of hepatic injury, based on Child-Pugh classification. In addition, the range of AUCs in cirrhotic patients, 28-412 ng·hr/ml, is higher than the range of AUCs in animal toxicity studies (42-127 ng·hr/ml). Therefore, the animal toxicity studies do not provide assurance of safety for rifaximin use in cirrhotic patients, particularly for those with more severe hepatic injury. The increased systemic exposure in cirrhotic patients is a cause for concern, given the limited absorption of rifaximin in animals and humans with normal liver function, the incidence of liver injury in toxicity studies that lacked toxicokinetic data, and the limited information about systemic toxicity. Furthermore, it will be difficult to monitor for rifaximin-induced liver injury in cirrhotic patients.

In order to address the safety concern about higher AUC values in cirrhotic patients, the Sponsor must conduct a chronic oral toxicology study that evaluates plasma AUC exposure in animals that is comparable to the highest plasma AUCs observed in cirrhotic patients (approximately 400 ng·hr/ml). These AUC values must be achieved and maintained throughout the duration of the chronic toxicity study. In the event that sufficiently high AUC levels cannot be achieved from oral dosing, alternative routes of administration may be used. The chronic toxicity study that is needed to address the safety concern about increased AUC values in cirrhotic patients may be conducted as a post-marketing study.

1.1.3 Labeling

Recommendations are shown below for the following subsections: "Pregnancy", "Carcinogenesis, Mutagenesis, Impairment of Fertility", and "Animal Toxicology and/or Pharmacology".

Sponsor's Proposed Version:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

There are no adequate and well controlled studies in pregnant women. XIFAXAN Tablets should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus. Rifaximin was teratogenic in rats at doses of 150 to 300 mg/kg (approximately 2.5 to 5 times the clinical dose for travelers' diarrhea [600 mg/day], adjusted for body surface area) (b) (4)

Evaluation: Although the proposed labeling is consistent with the current approved labeling for Xifaxan® with the single indication of treatment of travelers' diarrhea, the appropriate animal/human dose ratios based on the clinical dose of 550 mg BID, for maintenance of remission of hepatic encephalopathy, should be added to the current label. Animal/human dose ratios incorporating both indications are shown below in the "Recommended Version" of the label.

Recommended Version:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

There are no adequate and well controlled studies in pregnant women. XIFAXAN Tablets should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus. Rifaximin was teratogenic in rats at doses of 150 to 300 mg/kg (approximately 2.5 to 5 times the clinical dose for travelers' diarrhea [600 mg/day], and approximately 1.3 to 2.6 times the clinical dose for maintenance of remission of hepatic encephalopathy [1100 mg/day], adjusted for body surface area). Rifaximin was teratogenic in rabbits at doses of 62.5 to 1000 mg/kg (approximately 2 to 33 times the clinical dose for travelers' diarrhea [600 mg/day], and approximately 1.1 to 18 times the clinical dose for maintenance of remission of hepatic encephalopathy [1100 mg/day], adjusted for body surface area). These effects include cleft palate, agnatha, jaw

shortening, hemorrhage, eye partially open, small eyes, brachygnathia, incomplete ossification, and increased thoracolumbar vertebrae.

Sponsor's Proposed Version:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Malignant schwannomas in the heart were significantly increased in male Crl:CD® (SD) rats that received rifaximin by oral gavage for two years at 150 to 250 mg/kg/day (doses equivalent to 2.4 to 4 times the recommended daily dose of 200 mg TID, based on relative body surface area comparisons). There was no increase in tumors in Tg.rasH2 mice dosed orally with rifaximin for 26 weeks at 150 to 2000 mg/kg/day (doses equivalent to 1.2 to 16 times the recommended daily dose, based on relative body surface area comparisons).

Rifaximin was not genotoxic in the bacterial reverse mutation assay, chromosomal aberration assay, rat bone marrow micronucleus assay, rat hepatocyte unscheduled DNA synthesis assay, or the CHO/HGPRT mutation assay. There was no effect on fertility in male or female rats following the administration of rifaximin at doses up to 300 mg/kg (approximately 5 times the clinical dose of 600 mg/day, adjusted for body surface area).

Evaluation: Although the proposed labeling is consistent with the current approved labeling for Xifaxan® with the single indication of treatment of travelers' diarrhea, the appropriate animal/human dose ratios based on the clinical dose of 550 mg BID, for maintenance of remission of hepatic encephalopathy, should be added to the current label. Since the intended treatment population will have varying degrees of liver damage, Section 13.2 should be added to include the incidence of hepatotoxicity in nonclinical studies. In addition, Section 13.2 should describe the differences in plasma drug levels from chronic nonclinical toxicity studies compared to plasma drug levels in cirrhotic patients. Animal/human dose ratios based on the recommended doses for both indications, and the recommended text for Section 13.2 are shown below in the "Recommended Version" of the label.

Recommended Version:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Malignant schwannomas in the heart were significantly increased in male Crl:CD® (SD) rats that received rifaximin by oral gavage for two years at 150 to 250 mg/kg/day

(b) (4)

[redacted]

(b) (4)

Rifaximin was not genotoxic in the bacterial reverse mutation assay, chromosomal aberration assay, rat bone marrow micronucleus assay, rat hepatocyte unscheduled DNA synthesis assay, or the CHO/HGPRT mutation assay. There was no effect on fertility in male or female rats following the administration of rifaximin at doses up to 300 mg/kg (approximately 5 times the clinical dose of 600 mg/day, and approximately 2.6 times the clinical dose of 1100 mg/day, adjusted for body surface area).

13.2 Animal Toxicology and/or Pharmacology

Oral administration of rifaximin for 3-6 months produced hepatotoxicity in rats (50 mg/kg/day) and dogs (100 mg/kg/day). However, plasma drug levels were not measured in these studies. Subsequently, rifaximin was studied at doses as high as 300 mg/kg/day in rats for 6 months and 1000 mg/kg/day in dogs for 9 months, and no signs of hepatotoxicity were observed. The upper end of the range of plasma AUC values from the 6 month rat and 9 month dog toxicity studies (42-127 ng·hr/mL) is lower than the upper end of the range of plasma AUC values in cirrhotic patients (28-412 ng·hr/mL). [see *Clinical Pharmacology (12.3)*]

1.2 Brief Discussion of Nonclinical Findings

The sponsor has conducted a full battery of nonclinical studies, which included repeat-dose toxicology studies of up to 26-weeks in rats and 39-weeks in dogs. The toxicokinetic data from a 26-week oral toxicity study in rats and a 39-week oral toxicity study in dogs show that rifaximin has variable, but overall low systemic absorption. Over the course of development of rifaximin, chronic oral toxicity studies were performed twice in rats and twice in dogs. There were discrepancies in toxicity between the repeated chronic studies within in each species, specifically in the histopathology results (primarily in the small intestine and liver). The cause of these conflicting results was not established, however it was not due to the different dose levels in the studies (i.e. toxicity occurred in studies that used a lower dose range). Although systemic exposure to rifaximin is low, one possible explanation for the discrepancies may be a variation in exposure levels between the different studies. Toxicokinetics were measured only in one chronic (26-week) rat study and one chronic (39-week) dog study. Minimal signs of toxicity were noted in these studies. The range of AUC values that occurred in these studies (42 to 127 ng·hr/ml) was generally lower than the mean AUC observed in cirrhotic patients (130 ± 78 ng·hr/ml, with a range of 28 to 412 ng·hr/ml). Therefore, the toxicity studies in animals do not provide assurance of safety for the use of rifaximin in cirrhotic patients, where increased systemic exposure is expected.

To assess cardiac safety, the sponsor conducted an *in vitro* study to test the effects of rifaximin on the hERG potassium channel expressed in human embryonic kidney cells. Rifaximin concentrations of ≥ 30 μM produced a statistically significant increase in

inhibition of the hERG channel. The IC_{50} for the inhibitory effect of rifaximin on hERG potassium current was estimated to be 100 μM.

2 Drug Information

2.1 Drug

Xifaxan®

2.1.1 CAS Registry Number (Optional)

80621-81-4

2.1.2 Generic Name

rifaximin

2.1.3 Code Name

L 105

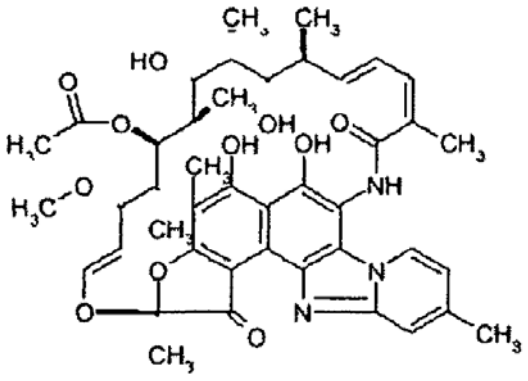
2.1.4 Chemical Name

(2S,16Z, 18E,20S,21S,22R,23R,24R,25S,26S,27S,28E)-5,6,21,23,25-pen-tahydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-2,7-(epoxypentadeca-[1,11,13]trien-imino)benzofuro[4,5-e]pyrido[1,2-α]-benzimidazole-1,15(2H)-dione,25-acetate

2.1.5 Molecular Formula/Molecular Weight

C_{43} H_{51} N_3 O_{11}/785.89

2.1.6 Structure



2.1.7 Pharmacologic class

Rifamycin Antibacterial

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

NDA 21,361 (traveler's diarrhea), (b) (4)

2.3 Clinical Formulation

immediate release 550 mg tablet

2.3.1 Drug Formulation

Table 1: Composition of Rifaximin Tablets, 550 mg

Component	Reference to Quality Standard	Function	mg/tablet
(b) (4)			
Rifaximin	In-house standard	Active ingredient	550
Sodium starch glycolate	NF	(b) (4)	(b) (4)
Glyceryl distearate (Glycerol palmitostearate)	NF		
Colloidal silicone dioxide	NF		
Microcrystalline cellulose	NF		
Talc	USP		
(b) (4)			
(b) (4)	USP		
	USP	(b) (4)	
Titanium dioxide	USP		
Propylene glycol	USP		
Red iron oxide	NF		
Disodium edetate	USP		
(b) (4)	USP	(b) (4)	
		Total Tablet Weight	**999**

[a] The qualitative and quantitative composition for (b) (4) are provided in (b) (4)
(b) (4) The components comply with their respective USP/NF monographs.

[b] Tablets are coated to an approximate weight gain of (b) (4) of the core tablet weight.

[c] (b) (4) is used as the solvent when mixing the coating solution. It is removed during the coating process.

2.3.2 Comments on Novel Excipients

None

2.3.3 Comments on Impurities/Degradants of Concern

None

2.4 Proposed Clinical Population and Dosing Regimen

Maintenance of remission of hepatic encephalopathy (HE) in patients 18 years of age and older. The proposed dosing regimen is 550 BID. (1100 mg/day).

2.5 Regulatory Background

XIFAXAN® (NDA 21,361) is approved for treatment of travelers' diarrhea at a dose of 200 mg t.i.d. for three days. Rifaximin was granted orphan designation for the treatment of hepatic encephalopathy, which encompasses the proposed indication as confirmed with the Office of Orphan Products Development on November 24, 2008. Rifaximin was submitted for priority review on June 24, 2009 (review period was extended due to a major amendment).

3 Studies Submitted

3.1 Studies Reviewed

Preclinical Study	Testing Laboratory	Study #	Lot #	Page
PHARMACOLOGY				
SAFETY PHARMACOLOGY				
Effect of Rifaximin on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	(b) (4)	090413.TBM	C8C0147	13
PHARMACOKINETICS/ TOXICOKINETICS				14
GENERAL TOXICOLOGY				16
GENETIC TOXICOLOGY				23

3.2 Studies Not Reviewed

The studies below are currently under review in the Division of Special Pathogen and Transplant Products:

"Rifaximin: Unscheduled DNA synthesis (UDS) in primary rat hepatocytes after *in vivo* treatment (Autoradiographic method)" [RTC 65100]

"Rifaximin: Immunotoxicity Study by Oral Gavage Administration to CD Rats for 28 days" [AFW 0041/063078]

3.3 Previous Reviews Referenced

Previous studies were reviewed under NDA 21,361 by Dr. Steven Kunder (review dated April 22, 2004).

"28-Day Repeated Oral Toxicity and Toxicokinetic Study in CByB6F1 Hybrid Mice with a Preliminary Range finding Toxicity Study: Analytical and Plasma Report" (AB41ZZ.2G3R (b) (4)) was reviewed under IND (b) (4) (Amendment #027 and #028 SX) (review by Dr. Tamal K. Chakraborti dated September 26, 2007).

The carcinogenicity studies were reviewed by Dr. Owen McMaster on December 7, 2009 (mouse) and December 18, 2009 (rat).

The review of a labeling supplement (NDA 21,361 (b) (4)) was completed by Dr. Steve Hundley (June 16, 2009) and Dr. William H. Taylor (December 22, 2009). In this supplement, the Sponsor requested that (b) (4).

4 Pharmacology

The following section contains summaries and excerpts from Dr. Steven Kunder's review of NDA 21,361 (April 22, 2004).

4.1 Primary Pharmacology

As a member of the rifampin class of antibiotics, rifaximin acts through binding to DNA-dependent RNA polymerase with inhibition of RNA synthesis.

4.2 Secondary Pharmacology

None

4.3 Safety Pharmacology

<u>Neurological Effects</u>

General behavioral effects were assessed in the Irwin test in male mice following oral administration of single doses of rifaximin (0, 100, 300, or 1000 mg/kg). No behavioral effects were seen during the 7-day monitoring period after dose administration.

Spontaneous activity was evaluated in ICR mice following single doses of 0, 100, 300, or 1000 mg/kg versus a positive control, diazepam (15 mg/kg). Rifaximin caused no effect on activity, while diazepam decreased activity over a one hour period post-dosing.

Hexibarbital-induced sleeping time was assessed using ICR mice given doses of 0, 100, 300, and 1000 mg/kg rifaximin, or the positive control, chlorpromazine (15 mg/kg). The onset of sleep time was noted after an intraperitoneal injection of hexabarbital, which was administered ~16 hours after initial dosing with either rifaximin or chlorpromazine. Rifaximin at 1000 mg/kg produced an increase in the time of sleep onset, while chlorpromazine prolonged sleep time. There was no effect at any other doses of rifaximin.

The proconvulsant activity of rifaximin was evaluated in male ICR mice at doses of 0, 100, 300, and 1000 mg/kg against metrazol-induced convulsions. A positive control, amphetamine sulphate (30 mg/kg), was also tested. In addition, the proconvulsant activity of rifaximin was evaluated in male ICR mice at doses of 0, 100, 300, and 1000 mg/kg against electroshock-induced convulsions. A positive control, bemegride (40 mg/kg), was also tested. None of the rifaximin doses showed any proconvulsant activity in either test.

Motor coordination was tested using the rotorod test. Female ICR mice were given doses of 0, 100, 300 and 1000 mg/kg rifaximin, or the positive control, mephenesin (400 mg/kg). There was no effect on motor coordination performance at an any dose of rifaximin, while mephenesin decreased performance.

Cardiovascular and Pulmonary Effects

In anesthetized cats, autonomic effects were studied at a dose of 1000 mg/kg. No effects were seen for basal blood pressure or heart rate, as well as nictitating membrane response. Responses to bilateral carotid occlusion and intravenous noradrenalin were not affected.

In anesthetized female Beagle dogs, intraduodenal administration of rifaximin (0 and 1000 mg/kg) was examined for cardiovascular and respiratory effects. No effects were seen on systolic, diastolic, mean arterial pressure, heart rate, left ventricular dp/dt maximum, electrocardiogram, femoral blood flow, femoral resistance, respiratory tidal volume, respiratory rate, and minute volume.

On September 17, 2009, the sponsor submitted the following safety pharmacology study, “Effect of Rifaximin on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells”. The review of the study report is shown below.

Effect of Rifaximin on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study #090413.TBM)

Methods: HEK293 (human embryonic kidney) cells were stably transfected with hERG potassium channel cDNA. The study protocol indicated that the cells were to be treated with 0 (vehicle), 10, 30, 100, or 300 μM Rifaximin for ~6 minutes (3 cells/group). The vehicle consisted of a HEPES-buffered physiological saline (HB-PS) solution (composition in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; Glucose,10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use), supplemented with 0.3% DMSO. Terfenadine (60 nM) was tested as a positive control compound. Cells stably expressing hERG were held at -80 mV. Onset and steady state inhibition of hERG potassium current due to Rifaximin were measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 sec; repolarizing test ramp to –80 mV (-0.5 V/s) repeated at 5 s intervals. In Phase I of the study, 10 μM Rifaximin was applied to cells in order to identify the approximate concentration range. In Phase II of the study, a concentration-response relationship was determined by applying a range of test article concentrations (30, 100, or 300 μM). Peak current was measured during the test ramp. A steady state was maintained for at least 20 s before applying test article or positive control. Peak current was measured until a new steady state was achieved. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition. The steady state before and after test article application was used to calculate the percentage of current inhibited at each concentration.

Results: The effect of Rifaximin on hERG channel function is shown in the following table.

Table 2: Mean Percent Inhibition of hERG Current by Rifaximin vs. Control

Rifaximin (μM)	***% Inhibition of hERG Current*** ***(Mean ± S.E.M.)***	***N***
Vehicle	*0.7 ± 0.1*	*3*
10	*6.7 ± 0.5*	*3*
30	*16.8 ± 1.0**	*3*
100	*34.5 ± 2.7**	*3*
300 (precipitation observed)	*44.3 ± 4.9**	*3*
60 nM Terfenadine (Stats vs. vehicle not provided)	*81.6*	*2*

* Value is statistically different than vehicle alone.

Rifaximin concentrations of ≥ 30 μM had a statistically significant increase in inhibition of the hERG channel. The IC_{50} for the inhibitory effect of Rifaximin on hERG potassium current is estimated to be 100 μM, given that precipitation occurred at 300 μM.

Renal Effects
Single doses of rifaximin (0, 100, 300, or 1000 mg/kg) or the diuretic, frusemide (20 mg/kg), were employed to determine the effect of rifaximin on urine volume and electrolyte excretion is Wistar rats. At the 1000 mg/kg dose, rats produced significantly increased urine output compared to animals given frusemide.

Gastrointestinal Effects
Motility effects of rifaximin were determined using the charcoal propulsion test in the ICR CD-1 mouse. Doses of 0, 100, 300, and 1000 mg/kg rifaximin, or a positive control, morphine sulphate, were tested. Morphine sulphate inhibited motility, while none of the doses of rifaximin had any effect on motility.

Gastric secretion effects were studied in male Wistar rats at doses of 0, 100, 300, and 1000 mg/kg rifaximin, or with a positive control, omeprazole (10 mg/kg). At doses up to 1000 mg/kg, rifaximin had no effect on volume or electrolyte content of gastric fluid, while omeprazole decreased H^+ and Na^+ content.

Gastrointestinal injury by rifaximin oral administration was examined in male Wistar rats at doses of 0, 100, 300, and 1000 mg/kg. A positive control, acetylsalicylic acid (200 mg/kg) was also tested. Acetylsalicylic acid produced significant gastric damage, while rifaximin had no effect.

5 Pharmacokinetics/ADME/Toxicokinetics

The following section contains summaries and excerpts from Dr. Steven Kunder's review of NDA 21,361 (April 22, 2004).

5.1 PK/ADME

Table 3: Multiple Dose Oral Pharmacokinetic Parameters in Rats

Parameter	26-week Rat Study (N2165) (N=9)					
	DAY 1			Week 26*		
DOSE (mg/kg/day)	50	150	300	50	150	300
$AUC_{(0\text{-}8\ hr)}$ (ng·hr/ml)	50.65	92.35	126.7	--	--	--

*Estimates of $AUC_{(0\text{-}8\ hr)}$ could not be made on week 26.

Table 4: Multiple Dose Oral Pharmacokinetic Parameters in Dogs

Parameter	39-week Dog Study (N2153) (N=8)					
	DAY 1			Week 39		
DOSE (mg/kg/day)	100	300	1000	100	300	1000
$AUC_{(0\text{-}8\ hr)}$ (ng·hr/ml)	41.75	102.1	66.05	61.35	75.45	105.7

PK/TK conclusions

In 26-week rat and 39-week dog pharmacokinetic studies, oral doses of rifaximin had a low level of systemic absorption. The range of C_{max} values was 6.4-25.0 ng/ml in rats and 9.3-21.9 ng/ml in dogs.

The results from ADME studies are as follows:

Absorption In rats, C14-rifaximin administered orally resulted in absorption of approximately 2% of the delivered dose by comparison of the exposure from intravenous dosing to oral dosing In dogs, systemic availability was approximately 12 %

Distribution Oral administration of C14-rifaximin in rats resulted in highest concentrations of radioactivity in the gastrointestinal tract, with approximately 67% in stomach/intestinal contents at 0 5 h, 78% at 1 0 h, 88% at 2 0 h, 47% at 4 h while tissue levels remained at less than 1% in any individual tissue

Metabolism Due to the low levels of systemic absorption, the sponsor did not characterize the metabolism of rifaximin

Excretion Intravenous administration of C14-rifaximin to dogs led to recovery of radioactivity in feces (83%), urine (6%), while oral administration resulted in 88% excretion in feces, 0 5 % in urine

The summary for the protein binding study below was provided by the Clinical Pharmacology reviewer (Dr. Insook Kim).

Protein Binding Report: *In Vivo* and *In Vitro* Plasma Protein Binding Study of Rifaximin by Ultrafiltration (Study #MC09M-0004)

Protein binding: Rifaximin is moderately protein bound, and the *in vivo* protein binding of rifaximin was about 9% lower in patients with hepatic impairment. In healthy subjects, the average protein binding ratio after administration of 550 mg rifaximin twice daily was 67.5% ranging from 62.5% to 72.8% with a coefficient of variation of 5.5%. Rifampin, a structural analog of rifaximin, is about 80% protein bound. In addition, the average ratio of protein binding in patients with hepatic impairment, after administration of 550 mg rifaximin twice daily, was 62.0% ranging from 55.3 to 68.2% with a coefficient of variation of 7.06%.

5.2 Toxicokinetics

N/A

6 General Toxicology

The following section contains summaries and excerpts from Dr. Steven Kunder's review of NDA 21,361 (April 22, 2004).

6.1 Single-Dose Toxicity

Selected acute toxicity studies are summarized below:

Acute studies were conducted with both oral and intravenous doses of rifaximin in different rodent species. In acute intravenous toxicity studies in mice and rats, animals

were dosed with a single dose of 40 mg/kg and observed for two weeks. In the mouse study, one mouse died within one hour of dosing (etiology of death unknown), and drug-treated mice showed clinical signs such as hunched posture, unsteady gait, respiratory distress, ptosis, pallid extremities, lethargy, tremors, cold body, and prostration. In the rat study, one rat died on dosing day 1 (etiology of death unknown), and drug-treated rats showed clinical signs such as respiratory distress, hunched posture, unsteady gait, increased activity, and tremors.

In an acute oral toxicity study in mice, animals were dosed with single doses of 1000 mg/kg (preliminary) and 2000 mg/kg (main study) and observed for two weeks. One mouse died on day 11 (2000 mg/kg). Mice treated with 2000 mg/kg showed piloerection and orange-colored feces indicative of large amounts of rifaximin. Similarly, an acute oral study was conducted in rats (single dose of 2000 mg/kg). All rats survived until necropsy and no adverse clinical signs other than piloerection and orange colored feces were noted. In an additional acute oral toxicity study, rats were dosed with a single dose of 500, 1000 or 2000 mg/kg, respectively. All rats survived until necropsy. In groups treated with 2000 mg/kg, hepatic steatosis was seen at necropsy.

6.2 Repeat-Dose Toxicity

Selected chronic toxicity studies are summarized below:

In a 13-week repeat-dose oral toxicity study (pre-GLP[1]) in rats, rifaximin was administered daily to male and female rats at doses of 0, 25, 50, and 100 mg/kg. Four rats died during the study, however, after microscopic examination, these deaths were attributed to non-drug related causes. After necropsy and microscopic examination, the 100 mg/kg group showed hepatic steatosis, hepatomegaly, mucosal ulceration, and slight nephrosis. No other drug-related clinical signs were noted and the no observed effect level (NOEL) was 50 mg/kg.

In a 26-week repeat-dose oral toxicity study (pre-GLP) in rats, rifaximin was administered at doses of 0, 25, 50 and 100 mg/kg. Four rats died during the study, however, these deaths were attributed to non-drug related causes. After histopathology examination, catarrhal enteritis was found in all treated groups. Hepatomegaly and infiltrate of connective cells into liver parenchyma and regression of renal parenchyma were observed in the 50 and 100 mg/kg groups. No other drug-related clinical signs were noted and the NOEL was 25 mg/kg.

The sponsor repeated the 26-week oral toxicity study in rats under GLP conditions, using dose levels of 0, 50, 150 and 300 mg/kg. There were no drug-related deaths. Histopathologic examination revealed no evidence of drug-induced lesions. However, all treatment groups exhibited lymphocytopenia, along with slight reductions in weight gain. Therefore, a NOAEL (no observed adverse effect level) was not established. The hematopoietic system is considered to be a target organ of toxicity at all dose levels.

[1] GLP: Good Laboratory Practices

The reduced lymphocyte counts were not of clinical significance, since there was no increase in the rate of infection in the treatment groups. Therefore, the tolerated dose is considered to be 300 mg/kg/day.

In a 26-week repeat-dose oral toxicity study (pre-GLP) in Beagle dogs, rifaximin was administered to male and female dogs at doses of 0 (lactose), 25, 50 and 100 mg/kg/day. All dogs survived through the end of the study, and body weight, food consumption, urinalysis, feces, and hematology were unaffected by drug treatment. The histopathology examination revealed some erosion of the intestinal mucosal membrane in drug-treated dogs. In addition, dogs dosed with 100 mg/kg/day exhibited hemostasis, mild degeneration in liver parenchyma, centrilobular fatty degeneration in the liver, and regression of renal parenchyma. Thus, due to the incidence of hepatotoxicity noted in the 100 mg/kg/day group, the tolerated dose in this study was 50 mg/kg/day. The incidence of hepatotoxicity was not stated in Dr. Steven Kunder's review of NDA 21,361. However, the Sponsor's original study report was consulted for this review, and upon reexamination of the histopathology data, I concluded that there was drug-related hepatotoxicity in the 100 mg/kg/day group.

In a 39-week oral toxicity study in dogs with a 4-week recovery period (GLP conditions), rifaximin was administered at doses of 0, 100, 300, and 1000 mg/kg/day. All dogs survived through the main study and recovery period, and no drug-related clinical signs were noted. Body weight, food consumption, ophthalmoscopy, and electrocardiography were unaffected by drug treatment. However, thymus weights of dogs treated with rifaximin were smaller than those of control dogs, and microscopic examination showed thymic involution and atrophy. These results were reversed following the 4-week recovery period. The toxicokinetic measurements showed that exposure increased with dose from the 100 to the 300 mg/kg dose, but was not increased proportionately at the 1000 mg/kg dose. A NOAEL was not achieved in this study due to the thymic effects.

The sponsor submitted the following repeat-dose toxicity study under IND (b) (4); "A 28-Day Repeated Oral Toxicity and Toxicokinetic Study in CByB6F1 Hybrid Mice with a Preliminary Range Finding Toxicity Study: Analytical and Plasma Report (AB41ZZ.2G3R (b) (4))." The review of the study report was taken from "Pharmacologist's review of IND (b) (4)" by Dr. Tamal K. Chakraborti, Ph.D. (September 26, 2007).

Study Title: 28-Day Oral (Gavage) Toxicity Study in CByB6F1 Mice

Key Study Findings: In a 28-day oral (gavage) toxicity study in CByB6F1 mice, animals were administered at 0 (1% w/v methylcellulose solution), 250, 1000, and 2000 mg/kg/day. There was no treatment-related mortality. Treatment with the test article did not produce any clinical signs of toxicity or have an adverse effect on body weights, hematology or clinical chemistry, gross pathology, organ weights. However, in males, body weight gain was reduced by 32.5% compared to control weight gain (1.06 g vs. 0.72 g) at 2000 mg/kg/day. In females, body weight gain was reduced by 13.27% compared to control weight gain (2.08 g vs. 1.88 g) at 2000 mg/kg/day. In males, food consumption was reduced by 13% at the high dose compared to control. However, in females, food consumption was not affected by treatment at any doses. The target organs of toxicity could not be identified in the absence of any significant histopathology findings in any organ or tissue. The MTD appears to be 2000 mg/kg/day based on reduction in body weight gain in both sexes.

Report No./Study No.: (b) (4) Study Number AB41ZZ.2G3R. (b) (4)

Volume #, and Page #: Volume 1, page 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: February 5, 2007

GLP Compliance: The compliance statement was not signed. This is a draft report.

QA Report: yes (X) no ( ).

Drug, Lot #, and % Purity: Rifaximin, Lot No. 0240241172, 98-101%

Formulation/Vehicle: 1% (w/v) methylcellulose in water

Methods:

Dosing:

Species/Strain: CByB6F1 mice

No./Sex/Group: 10/sex/group

Satellite Groups Used for Toxicokinetics: 20/sex/group (5/sex for control)

Age: 6-8 weeks

Weight: Males: 20.4-26.8 g; Females: 15.0-21.3 g

Doses in Administered Units: 0, 250, 1000, and 2000 mg/kg/day. Dose selection was based on the preliminary 5-day range-finding portion of the study, where male and female mice (n = 5/sex/group) were dosed orally with 0 (1 % methylcellulose) or Rifaximin at 125, 250, 500, 1000 and 2000 mg/kg/day ((b) (4) Study Number AB41ZZ.2G3R. (b) (4). Parameters evaluated included clinical signs and body weight. There were no deaths, no clinical signs and no body weight effects.

Route, Form, and Volume: Oral gavage, suspension, and 15 ml/kg

Observations and Times:

Clinical Signs: Clinical signs were observed twice daily.

Body Weights: Body weights were recorded once a week.

Food Consumption: Food consumption was recorded on a weekly basis.

Hematology: Hematology was conducted at necropsy.

Clinical Chemistry: Serum chemistry was conducted at necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organs weighed: The following organs were weighed from all main study animals: brain, heart, liver, kidneys, spleen, adrenal and testes/ovaries.

Histopathology: The following organs/tissues were examined for histopathology from all main study animals: adrenal, aorta, bone (femur and sternum), bone marrow (femur and sternum), brain , epididymides, esophagus, eyes, gall bladder, gross lesions, Harderian gland, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs and bronchi, lymph nodes (mesenteric and mandibular), mammary gland, nasal cavity, ovaries, pancreas, parathyroid glands, prostate, salivary, sciatic nerve, seminal vesicles, skeletal muscles (thigh), small intestine (duodenum, jejunum and ileum), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus and vagina.

Toxicokinetics: Eighteen TK animals per sex and dose (3/sex/dose/timepoint) were bled after their last dose in the fourth study week and after 27 days of daily treatments. Test article-treated animals were bled at 0.5, 1, 2, 4, 8, and 24 hours after the last dose administration. Vehicle control animals were bled 1 hour after the last dose administration. Prior to bleeding, the animals were anesthetized with CO2/02 and bled from the retro-orbital sinus.

Results:

Mortality: One control female died on Day 5. There was no other mortality.

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

Body Weights: The mean initial and final (Week 4) body weight of the control males was 23.21 g and 21.88 g, respectively. The mean initial and final (Week 4) body weight of control females was 18.51 and 18.39 g, respectively. In males, body weights were 103, 102 and 99% of control at 250, 1000 and 2000 mg/kg/day, respectively. In females, body weights were 98%, 102% and 103% of control at 250, 1000 and 2000 mg/kg/day, respectively. In males, body weight gain was reduced by 32.5% compared to control weight gain (1.06 g vs. 0.72 g) at 2000 mg/kg/day. In females, body weight gain was reduced by 13.27% compared to control weight gain (2.08 g vs. 1.88 g) at 2000 mg/kg/day.

Body weight (g) of males

Treatment (mg/kg/day)	Initial	Week 4	% of Control (Week 4)	Weight Gain (Final-Initial) (g)	Change (% of Control)
0	23.21	24.27	100	1.06	100
250	23.36	25.06	103.26	1.70	159
1000	23.17	24.89	102.55	1.72	162
2000	23.35	24.07	99.18	0.72	67.5 (32.5%)*

* **():** Reduction in body weight gain compared to control

Body weight (g) of females

Treatment (mg/kg/day)	Initial	Week 4	% of Control (Week 2)	Weight Gain (Final-Initial) (g)	Change (% of Control)
0	18.51	20.59	100	2.08	100
250	17.85	20.11	97.67	2.26	112.67
1000	18.83	20.96	101.80	2.13	100.66
2000	19.29	21.17	102.82	1.88	86.73 (13.27%)*

* **():** Reduction in body weight gain compared to control

Food Consumption: The mean initial and final food consumption in control males were 0.75 and 0.60 g/animal/day, respectively. The mean initial and final food consumption control females were 0.64 and 0.59 g/animal/day, respectively. There were no significant

treatment-related changes. In males, final food consumption value was 87% of control at the high dose. In females, the final food consumption was 99% of control at the high dose.

Organ Weights: No significant treatment-related effects were observed.

Hematology: There were no significant treatment-related changes.

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

Gross Pathology: No gross lesions considered to be test article related were noted in any animal of either sex in any dose group during necropsy.

Histopathology: There were no significant treatment-related changes.

Toxicokinetics: As the dose increased 8-fold from 250 mg/kg/day to 2,000 mg/kg/day, there was no apparent increase in Cmax or $AUC_{0\text{-}24hr}$ for male mice indicating a constant systemic exposure throughout the dose range. The Cmax and $AUC_{0\text{-}24hr}$ values for female mice were consistently higher than the corresponding values for male mice at each dose level. The Cmax values were similar for female mice receiving the low and mid doses and higher for the animals receiving the highest dose. The $AUC_{0\text{-}24hr}$ values increased with increasing dose for female mice, but the overall increase was only 1.8-fold for the 8-fold increase in dose from 250 and 2,000 mg/kg/day. For male mice, Tmax was observed at 0.5, 1 or 2 hr at 250, 1000 and 2000 mg/kg/day, respectively. For female mice, Tmax at 1,000 mg/kg/day occurred at 8 hr, but for animals receiving 250 or 2,000 mg/kg/day, Tmax was observed at 0.5 or 1 hr, respectively. The following table (from page 2 of the Vol. 1) shows the mean TK parameters.

Table 1. Toxicokinetic Data from Day 27 of the Mouse Study

Parameters	Dose (mg/kg/day)					
	250		1000		2000	
	M	F	M	F	M	F
C_{max} (ng/mL)	10.2	15.0	9.78	13.1	11.8	25.8
$AUC_{0\text{-}24}$ (ng•hr/mL)	95.9	117	69.6	176	88.4	215
T_{max} (hr)	2	0.5	1	8	0.5	1
$T_{½}$ (hr)	22.3*	5.7*	14.2	13.5*	10.0	22.3*

* Considered unreliable as the correlation coefficient was less than 0.8.

Summary: In a 28-day oral (gavage) toxicity study in CByB6F1 mice, animals were tested at 0 (1% w/v methylcellulose solution), 250, 1000, and 2000 mg/kg/day. There was no treatment-related mortality. Treatment with the test article did not produce any clinical signs of toxicity or have an adverse effect hematology or clinical chemistry, gross pathology, organ weights. However, in males, body weight gain was reduced by 32.5% compared to control weight gain (1.06 g vs. 0.72 g) at 2000 mg/kg/day. In females, body weight gain was reduced by 13.27% compared to control weight gain (2.08 g vs. 1.88 g) at 2000 mg/kg/day. In males, food consumption was reduced by 13% at the high dose compared to control. However, in females,

food consumption was not affected by treatment at any doses. The target organs of toxicity could not be identified in the absence of any significant histopathology findings in any organ or tissue.

7 Genetic Toxicology

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

Rifaximin was not genotoxic in the bacterial reverse mutation assay.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Rifaximin was not genotoxic in the chromosomal aberration assay.

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

Rifaximin was not genotoxic in the rat bone marrow micronucleus assay.

7.4 Other Genetic Toxicity Studies

Rifaximin was not genotoxic in the CHO/HGPRT mutation assay.

8 Carcinogenicity

On April 8, 2009, the sponsor submitted complete study reports from a 26-week oral carcinogenicity study of rifaximin in Tg.rasH2 mice and a 2-year oral carcinogenicity study in rats to the Division of Special Pathogen and Transplant Products (DSPTP). The attached reviews of the carcinogenicity studies were completed by Dr. Owen McMaster on December 7, 2009 (mouse) and December 18, 2009 (rat).

Study Title	26-week repeated dose oral carcinogenicity study of rifaximin in Tg.rasH2 mice
BioReliance Study no	AB41ZZ.7G8R (b) (4)
Report no.	RFTK1002
Study report location:	IND (b) (4) volume 90.1-2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 5, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug lot numbers	0240241172 (CSC0229) and C7G0217
Drug purity	100.2 % and 99.2 %
Key study findings	Rifaximin administration at doses of 150, 500 and 1500 mg/kg/day (males) and 250, 750 and 2000 mg/kg/day (females) did not increase the incidence of tumors in Tg.rasH2 mice.
Adequacy of carcinogenicity study	The study was adequate to evaluate the oncogenic potential of rifaximin. The study protocol was approved by the Exec CAC on October 9, 2007.
Appropriateness of test model	The test model was appropriate. Constitutively active ras mutations are commonly found in human neoplasms and the Tg.rasH2 mouse model has 5 to 6 copies of the prototype human c-Ha-ras oncogene inserted along with its own promoter and enhancer (Morton et al: (2002) The Tg.rasH2 Mouse in Cancer Hazard Identification. *Toxicologic Pathology* 30:139–146). The mechanism of carcinogenesis is therefore relevant to humans. This model is recommended in ICH Harmonized guideline S1B: Testing for Carcinogenicity of Pharmaceuticals and the protocol was approved by the Exec CAC.
Evaluation of tumor findings	Rifaximin administration for six months was not associated with an increase in the incidence of tumors in Tg.rasH2 mice.
ECAC meeting minutes	N/A
Methods	
Doses	Males: 150, 500 and 1500 mg/kg/day, Females: 250, 750 and 2000 mg/kg/day.

Basis of dose selection	Dose levels were selected based on previous studies and were approved by the Exec CAC
Species	Mouse (*Mus musculus*)
Strain	Tg.rasH2 or CByB6F1 (controls)
Number/sex/group	25
Route	Oral gavage
Formulation	Rifaximin was prepared in 1 % methylcellulose, 400 centipoises (cps) in deionized water.
Dose volume	15 mL/kg
Frequency of dosing	Once daily
Satellite groups	8 mice/sex were dosed for pharmacokinetics studies. Six vehicle control mice (3/sex) and six rifaximin-treated mice were bled for pharmacokinetics evaluations 1 hour after dosing on each of two the bleed days during Week 13 and Week 26.
Age	Approximately 7 weeks of age
Animal housing	Animals were housed individually.
Dosing solution analyses	Concentration analyses were conducted during Weeks 1, 7, 8, 13, 17, 21 and 25 and showed that dosing solutions were prepared accurately and generally met the acceptance criteria for recovery (b) (4). Actual concentrations ranged from (b) (4) of target. Samples that did not meet the acceptance criteria were (b) (4) of target. Week 1 data are shown on Table 3 below.
Homogeneity of Dosing formulations	The homogeneity samples met the acceptance criteria for precision. Percentage relative standard deviation values ranged from 2.62 to 9.34 %. See Table 2 for the results of homogeneity analyses.
Dual controls employed	No

Table 1: Experimental Design

			Number of animals/group	
Group #	Sex	Rifaximin Dose (mg/kg/day)	Main study	pK study
1	M,F	0 (vehicle)	25/sex	8/sex
2	M	150	25	8
3	F	250	25	8
4	M	500	25	8
5	F	750	25	8
6	M	1500	25	8
7	F	2000	25	8
8	M	0 (urethane) [4]	25	
9	F	0 (urethane) [4]	25	

Positive control animals received a total of 3 intraperitoneal injections of urethane (1000 mg/kg, 10 mL/kg, and one injection on each of Days 1, 3 and 5.

Table 2: Homogeneity analysis of dosing formulations.

Dose level (mg/kg/day)	Nominal Concentration (mg/mL)	Average Calculated Concentration (mg/mL)	Average recovery (%)	% Relative standard deviation
150 250 500 750 1500 2000	(b) (4)			

Each data point represents an average of seven samples each.

Concentration Analysis

The concentration samples met the acceptance criteria for accuracy ((b) (4) percent recovery [PR]) with a few exceptions. Table 3 shows the time points, doses and percent recovery for the samples that did not meet the acceptance criteria.

Table 3: Percent recovery for the samples that did not meet the acceptance criteria

Time point	Dose group	Average PR
Week 1	2000 mg/kg/day	(b) (4)
Week 21	150 mg/kg/day	
Week 21	500 mg/kg/day	

Observation times and Results

Mortality

Mortality/moribundity was evaluated two times daily. There were no statistically significant increases in mortality/moribundity, when rifaximin-treated animals were compared to controls. There were no deaths in the male or female vehicle controls and 3, 0 and 1 death/moribund sacrifices in the low-, mid- and high-dose females. There was 1 moribund sacrifice in the low-dose males and one death in the mid-dose males but no mortality at the high dose. See Table 4 below for details. The sponsor considered the deaths to be rifaximin-related since gross necropsy or histopathology evaluation did not show any signs of gavage error. No cause of death was provided.

Table 4: Mortality/moribundity in rifaximin-treated animals compared to positive and negative controls

MALES

	Cause of Death	Group 1	Group 2	Group 4	Group 6	Group 8
Day 32	Moribund Sacrifice	-	1/25	-	-	-
Day 64	Natural Death	-	-	-	-	1/25
Day 93	Natural Death	-	-	-	-	2/24
Day 99	Natural Death	-	-	-	-	3/22
Day 103	Natural Death	-	-	-	-	1/19
Day 106	Natural Death	-	-	-	-	1/18
Day 113	Natural Death	-	-	-	-	1/17
Day 115	Natural Death	-	-	-	-	2/16
Day 117	Natural Death	-	-	-	-	1/14
Day 118	Natural Death	-	-	-	-	1/13
Day 120	Natural Death	-	-	-	-	1/12
Day 121	Scheduled Sacrifice	-	-	-	-	11/11
Day 190	Natural Death	-	-	1/25	-	-
Day 190	Terminal Sacrifice	25/25	-	-	25/25	-
Day 191	Moribund Sacrifice	-	-	-	-	-
Day 191	Terminal Sacrifice	-	24/24	24/24	-	-
	Total	**25/25**	**25/25**	**25/25**	**25/25**	**25/25**

FEMALES

	Cause of Death	Group 1	Group 3	Group 5	Group 7	Group 9
Day 98	Natural Death	-	-	-	-	1/25
Day 106	Natural Death	-	1/25	-	-	-
Day 111	Natural Death	-	-	-	-	1/24
Day 113	Natural Death	-	-	-	-	2/23
Day 115	Natural Death	-	-	-	-	1/21
Day 119	Scheduled Sacrifice	-	-	-	-	20/20
Day 160	Moribund Sacrifice	-	1/24	-	-	-
Day 170	Moribund Sacrifice	-	-	-	1/25	-
Day 176	Moribund Sacrifice		1/23			
Day 190	Terminal Sacrifice	25/25	-	-	24/24	-
Day 191	Terminal Sacrifice	-	22/22	25/25	-	-
	Total	**25/25**	**25/25**	**25/25**	**25/25**	**25/25**

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 150 mg/kg/day Group 3 - 250 mg/kg/day
Group 4 - 500 mg/kg/day Group 5 - 750 mg/kg/day Group 6 - 1500 mg/kg/day
Group 7 - 2000 mg/kg/day Group 8 or 9 - 1000 mg/kg/day Urethane

Note: Represents the number of animals affected / the number of animals remaining on test.

Clinical signs

Test animals were also observed (cage side) for clinical signs of toxicity within two hours after dosing. Detailed clinical evaluations were conducted weekly. The incidence of test-article-related clinical signs was lower in males than in females receiving similar doses. Males showed ruffled fur, rapid, shallow breathing and hyperactivity, while females showed ruffled fur, skin abnormality, decreased motor activity, dyspnea, cowering, desquamation, stained coat, palpable mass and hunched appearance. These findings occurred with low incidence and did not follow a dose response.

Body weights

Bodyweights were measured weekly through Week 13 and then biweekly thereafter. No biologically significant test article-related effects on bodyweights were seen during the study. Slight increases and decreases were recorded during the study but due to lack of consistency, rifaximin was not considered to have any effect on body weight.

Food consumption

There were no test article-related effects on mean food consumption values. Despite sporadic increases and decreases in weekly food consumption values which were significantly different from controls, these changes were not considered toxicologically significant.

Histopathology findings-Non-neoplastic

Acute exudative inflammation of the nasal cavity was more severe in test article-treated mice compared to vehicle-treated mice (see Table 5 below). This finding was characterized by the presence of eosinophilic protein rich material that was infiltrated by degenerate neutrophils, erythrocytes, necrotic debris, mucin and fibrin in the lumen of the nasal cavities. The pathologist did not propose a cause for this finding. The pathologist concluded that none of the other non-neoplastic findings were attributable to rifaximin because of low incidence, lack of dose dependency and/or the presence of similar findings in control animals.

Table 5: Incidence of nasal cavity lesions in male and female mice treated

	Males				Females			
Groups	1	2	4	6	1	3	5	7
Number Examined	25	25	25	25	25	25	25	25
Inflammation, submucosa, minimal	13	10	9	8	19	13	9	10
Inflammation, exudative	0	0	0	0	0	0	0	0
minimal	3	0	0	2	0	0	0	0
mild	0	0	0	6	0	0	0	0
moderate	0	0	0	2	0	0	0	0
Inflammation, acute, exudative	0	0	0	0	0	0	0	0
minimal	0	0	0	0	3	0	3	2
mild	0	0	0	0	0	0	0	4
moderate	0	0	0	0	0	0	0	3

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 150 mg/kg/day Group 3 - 250 mg/kg/day
Group 4 - 500 mg/kg/day Group 5 - 750 mg/kg/day Group 6 - 1500 mg/kg/day
Group 7 - 2000 mg/kg/day

Histopathology findings-Neoplastic

Target organs

Lung

The incidence of all pulmonary tumors was not significantly increased in rifaximin-treated mice compared to controls (see Table 6). In males, the incidence of pulmonary tumors was either very low or similar across all groups. In females, the number of lung tumors in rifaximin-treated

animals was greater than the number in vehicle controls, but statistical analysis showed no dose response relationship. Also, the highest incidence of pulmonary adenomas in this study (4/25) was only just outside the range of historical controls (up to 3/25, see Table 7). In contrast, the urethane-treated positive control mice of both sexes had a significantly higher incidence of pulmonary tumors.

Table 6: Incidence of pulmonary tumors in TgrasH2 mice treated with rifaximin or urethane

	Males					Females				
Groups	1	2	4	6	8	1	3	5	7	9
Number Examined	25	25	25	25	25	25	25	25	25	25
Single adenoma	2	3	3	1	0	1	2	4	3	0
Multiple adenoma	0	0	0	1	25*	0	0	1	0	25*
Carcinoma	1	0	0	0	18*	0	0	0	0	24*
Number of animals with at least one type of lung tumor	3	3	3	2	25	1	2	5	3	25

Note: Multiple adenomas and/or carcinomas were present in the same animal in urethane treated mice of both sexes.
* $p<0.05$, Fisher's Exact Test Compared to Group 1.
The Cochran-Armitage Test was negative for a dose response.

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 150 mg/kg/day Group 3 - 250 mg/kg/day
Group 4 - 500 mg/kg/day Group 5 - 750 mg/kg/day Group 6 - 1500 mg/kg/day
Group 7 - 2000 mg/kg/day Group 8 or 9 - 1000 mg/kg/day Urethane

Table 7: Historical control data: Incidence of pulmonary adenomas in vehicle-treated mice

Study number	Males	Females
AA71US.7G8R.(b) (4)	3/25	2/25
AA74UX-UY.7G8R.(b) (4)	1/25	2/25
AA8OUM.7V8R.(b) (4)	1/25	0/25
ABO3CL.7G8R.(b) (4)	4/25	2/25
AA98KN.7G8R.01.(b) (4)	1/25	2/25
AB28EM.7G8R(b) (4)	3/25	2/25
AB37CC.7G8R.	3/25	3/25
ACOO8H.7G8R	2/25	1/25

Spleen

The incidence of splenic hemangiosarcomas was sporadically increased in rifaximin-treated mice compared to controls (see Table 8), but the trend analysis was negative for a dose response.

Urethane-treated positive control animals showed a statistically significant increase in splenic hemangiosarcomas.

Table 8: Incidence of splenic hemangiosarcomas in rifaximin-treated TgrasH2 mice

	Males					Females				
Groups	1	2	4	6	8	1	3	5	7	9
Number Examined	25	25	25	25	25	25	25	25	25	25
Hemangiosarcoma	0	1	4	0	20*	0	1	1	2	25*

* $p<0.05$, Fisher's Exact Test Compared to Group 1.
The Cochran-Armitage Test was negative for a dose response.
Trend analysis (Regression Analysis) negative for a dose response.

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 150 mg/kg/day Group 3 - 250 mg/kg/day
Group 4 - 500 mg/kg/day Group 5 - 750 mg/kg/day Group 6 - 1500 mg/kg/day
Group 7 - 2000 mg/kg/day Group 8 or 9 - 1000 mg/kg/day Urethane

Table 9: Incidence of hemangiosarcomas in organs other than the spleen.

	Males				Females			
Groups	1	2	4	6	1	3	5	7
Number Examined	25	25	25	25	25	25	25	25
Cecum	0	0	0	0	0	1	0	0
Kidney	0	1	0	0	0	0	0	0
Liver	0	1	0	0	0	0	0	0
Lungs	0	0	0	3	0	0	0	1
Pancreas	0	0	0	0	0	1	0	0
Salivary glands	0	0	1	0	0	0	1	0
Skeletal muscle	0	0	0	0	0	1	0	0
Subcutis	0	0	0	0	0	0	1	0
Testes	1	0	0	0	0	0	0	0
Urinary bladder	0	0	0	0	0	1	0	0
Uterus	0	0	0	0	1	0	0	1
Incidence of all Hemangiosarcomas	1	2	1	3	1	4	2	2

Fisher's Exact Test did not reveal any significant differences when the test article-treated groups were compared to the vehicle control group (Group 1).
The Cochran-Armitage Test was negative for a dose response.
Trend analysis (Regression Analysis) was negative for a dose response.

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 150 mg/kg/day Group 3 - 250 mg/kg/day
Group 4 - 500 mg/kg/day Group 5 - 750 mg/kg/day Group 6 - 1500 mg/kg/day
Group 7 - 2000 mg/kg/day

Pharmacokinetics

Table 10: Mean plasma rifaximin concentrations (ng/mL) in Tg.rasH2 mice

Dose (mg/kg/day)	Mean Plasma Concentrations (ng/mL)							
	Males				Females			
	0	150	500	1500	0	250	750	2000
Week 13	BLQ	6.84 ± 1.95	6.52 ± 0.99	11.5 ± 8.62	0.45 ± 0.79	10.5 ± 1.82	15.3 ± 4.75	17.7 ± 5.90
Week 26	0.51 ± 0.89	5.03 ± 0.40	8.40 ± 1.84	14.4 ± 5.98	BLQ	9.17 ± 0.30	12.3 ± 2.72	17.3 ± 7.42

In humans and in animals rifaximin is poorly absorbed systemically from the gastrointestinal tract. In this study, rifaximin plasma levels generally increased with increasing dose but the increases were much less than dose-proportional. In fact, in the male mice during week 26, a 10-fold increase in the administered dose of rifaximin (150 vs. 1500 mg/kg/day) only resulted in ~ 3-fold increase in plasma concentration (14.4 compared with 5.03). The considerable overlap of the standard deviations of the mid- and high-doses suggests that we on a flat or flattening portion of the dose response curve, a feature which may diminish the significance of a trend analysis.

Discussion

This study was adequate and conducted based on a protocol approved by CDER Executive Carcinogenicity Assessment Committee. Exposure was confirmed by drug plasma measurements but the increase in plasma rifaximin was less than proportional to the increase in rifaximin dose. The Tg.rasH2 model was sensitive and mice responded appropriately to the positive control urethane with statistically significant increases in multiple adenomas, carcinomas and hemangiosarcomas.

In females, the number of (single) lung adenomas in rifaximin-treated mice (2/25, 4/25 and 3/25) was greater than the number in vehicle controls (1/25), but statistical analysis using the Cochran-Armitage test did not detect a dose response trend. However, the relatively flat exposure-response curve may mask a dose-response trend.

Historical incidence of pulmonary adenomas in vehicle treated females was listed as 7 % but this number was calculated as [total number of mice affected]/ [total number of mice used in all the studies or 14/200].The data from the individual studies (see Table 7) show that as many as 3/25 vehicle-treated mice in one study (Study B37CC.7G8R.(b) (4)) had single adenomas. In the present study only Group 5 had a slightly higher incidence (4/25) and the incidence at the highest dose (3/25) was comparable to the data from historical controls. This carcinogenicity study was therefore deemed negative due to the absence of a positive dose response trend and because tumor incidence was only slightly outside of the historical control range.

Conclusion

Rifaximin administration to Tg.rasH2 mice for six months did not result in a statistically significant increase the incidence of tumors at 150, 500 and 1500 mg/kg/day (males) or 250, 750 or 2000 mg/kg/day (females).

Study Title	Rifaximin: A 2-year oral carcinogenicity study in rats
Study no	1310-001
Study report location:	DARRTS SDN 197
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 5, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug lot numbers	0020340370 and C7G0217
Drug purity	100.4 % and 99.2 %
Key study findings	Rifaximin administration resulted in a statistically significant increase in the incidences of malignant schwannoma in the heart of male rats.
Adequacy of carcinogenicity study	The study was adequate to evaluate the oncogenic potential of rifaximin. The study protocol was approved by the Exec CAC.
Appropriateness of test model	The test model was appropriate and approved by the Exec CAC.
Evaluation of tumor findings	Rifaximin administration for two years was associated with an increase in the incidence of malignant schwannoma in heart of male rats.

Doses

Low dose	20 mg/kg
Mid dose	50 mg/kg
High dose	150 mg/kg (Week 0-27)
	200 mg/kg (Week 28-38)
	250 mg/kg (Week 39-104)

Basis of dose selection	Dose levels were selected based on MTD (reduction of body weight gain in CD rats from 4- and 26-week oral (gavage) studies) and approved by the Exec CAC
Species	Rat (*Rattus norvegicus*)
Strain	CD®[Crl:CD® (SD)]
Number/sex/group	60
Route	Oral gavage
Formulation	Rifaximin was prepared in 1 % methylcellulose, 4000 centipoises in deionized water.
Dose volume	10 mL/kg/dose
Frequency of dosing	Once daily
Satellite groups	N/A
Age	Approximately 6 weeks of age
Animal housing	Animals were housed individually.
Dietary restriction paradigm	Meal Lab Diet® was available *ad libitum*
Dosing solution analyses	Concentration analyses showed the solutions were accurately prepared (See Table 5). Formulations were prepared weekly and refrigerated when not in use. See Table 4 for sample collection schedule.
Drug stability/homogeneity	Dosing formulations were determined to be stable and homogeneous. See Table 6 and 7 for the results of stability and homogeneity analyses.

Dual controls employed No

[1] Deviations from original protocol

(1) The protocol was approved after the animals arrived at (b) (4) The initial animal receipt data and initial cage side observations were therefore collected prior to the approval of the protocol.

(b) (4)

The applicant therefore requested (January 18, 2007) that the high dose be increased to 200 mg/kg and this request was granted by DGP. At Week 28, the high dose was therefore increased to 200 mg/kg and at Week 39, the high dose was increased to 250 mg/kg for the remainder of the study.

Observation times and Results

Mortality

Mortality/moribundity was evaluated two or three times daily. No test article related differences on mortality were detected and survival was similar among the groups. See Table 1and Figure 1

Clinical signs

Clinical signs were evaluated weekly. There were no test article-related clinical findings. Clinical findings noted were common to laboratory rats.

Body weights

Bodyweights were measured weekly for the first 14 weeks and then biweekly thereafter. No significant test article-related effects on bodyweights were seen during the study. See Table 2.

Food consumption

There were no test article-related effects on mean food consumption values. Despite sporadic weekly food consumption values which were significantly different from controls, these changes were not considered toxicologically significant. See Figures 3 and 3A.

Histopathology findings-Non-neoplastic

The pathologist concluded that none of the non-neoplastic findings were attributable to rifaximin. All microscopic findings were considered incidental and not related to rifaximin administration due to low incidence, lack of dose dependency and/or the presence of similar findings in control animals.

Histopathology findings-Neoplastic

Neoplasms were detected in several tissue types in rifaximin-treated and control animals. These

data were subjected to statistical analysis by FDA statistician Mohammad Atiar Rahman, Ph.D. from the Division of Biometrics -6. Please see Table 3 and attached Statistics review for details.

There were statistically significant positive dose response relationships in the incidences of malignant schwannoma in heart and islet cell carcinoma in pancreas in male rats. However, none of the pair wise comparisons were found to be statistically significant. See Table 3 (taken from Statistician's review).

A subsequent evaluation of historical control neoplastic data from (b) (4) (where the study was conducted) revealed that the incidence of islet cell carcinoma in pancreas in male rats in this study (5 %) was within the limits of the historical control data (up to about 7 %). On the other hand, the incidence of malignant schwannoma in heart (5 %) was outside the range of historical controls (up to 1.7 %) in addition to having a statistically significant positive dose response relationship in the present study.

Discussion

This study was adequate and conducted based on a protocol approved by the CDER Executive Carcinogenicity Assessment Committee. Based on interim data, the sponsor requested and received permission from the Division of Gastroenterology Products, to increase the high dose to 200 mg/kg and then to 250 mg/kg. Survival and bodyweight data were comparable between the dose groups. Study design could have been improved by the use of dual controls and Peer review.

Rifaximin administration to male rats for two years was associated with a statistically significant positive dose response relationship in the incidence of malignant schwannoma in the heart. The sponsor acknowledged the statistically significance of the finding, but did not consider this finding to be related to rifaximin because the overall incidence was low, the pair-wise test was not statistically significant, there were no similar findings in female rats, and schwannomas have been detected in control animals.

After an initial review of the data submitted, this reviewer requested that the applicant submit historical control neoplasm data from (b) (4) the company that conducted the carcinogenicity study. A review of these data revealed that, although malignant schwannomas have been detected in control animals, the incidence in the present study (5 %) was outside the range of the historical control data provided (up to 1.7 %). This reviewer therefore concluded that although none of the pairwise comparisons were found to be statistically significant, this increase must be considered to be biologically significant because the dose response relationship was statistically significant and the tumor incidence was outside of the limits of the historical control data. This dose is about 2.3-times the clinical dose (2x550mg/day) based on body surface area comparisons.

This increase in malignant schwannomas is consistent with the tumor findings with the other rifamycins. RIFADIN's prescribing information describes its association with "a few cases of accelerated growth of lung carcinoma" in man and an increased incidence of hepatomas in female mice. PRIFTIN ® prescribing information describes its association with hepatocellular carcinomas in mice and nasal cavity adenomas in rats.

Despite a statistically significant dose response relationship in the incidences of islet cell carcinoma, the pairwise comparisons were not found to be statistically significant and the incidence was within the range of historical controls.

Table 1. Quarterly survival of rats in Study 1310-001: Rifaximin: A 2-year oral carcinogenicity study in rats

The quarterly survival of each group during the study is provided in the following table.

Survival - Males									
	Weeks								
Males	**-1**	**13**	**26**	**38**	**52**	**64**	**78**	**92**	**Term**
Vehicle Control	60	60	60	60	57	54	48	34	16
20 mg/kg/day	60	59	59	58	56	52	45	34	15
50 mg/kg/day	60	60	60	58	55	53	47	34	20
150/200/250 mg/kg/day	60	60	60	59	56	49	42	28	15

Survival - Females									
	Weeks								
Females	**-1**	**13**	**26**	**38**	**52**	**64**	**78**	**92**	**Term**
Vehicle Control	60	60	60	59	58	57	54	43	29
20 mg/kg/day	60	60	59	59	59	58	47	35	25
50 mg/kg/day	60	60	57	57	56	54	46	40	28
150/200/250 mg/kg/day	60	60	58	58	57	55	47	37	24

Figure 1

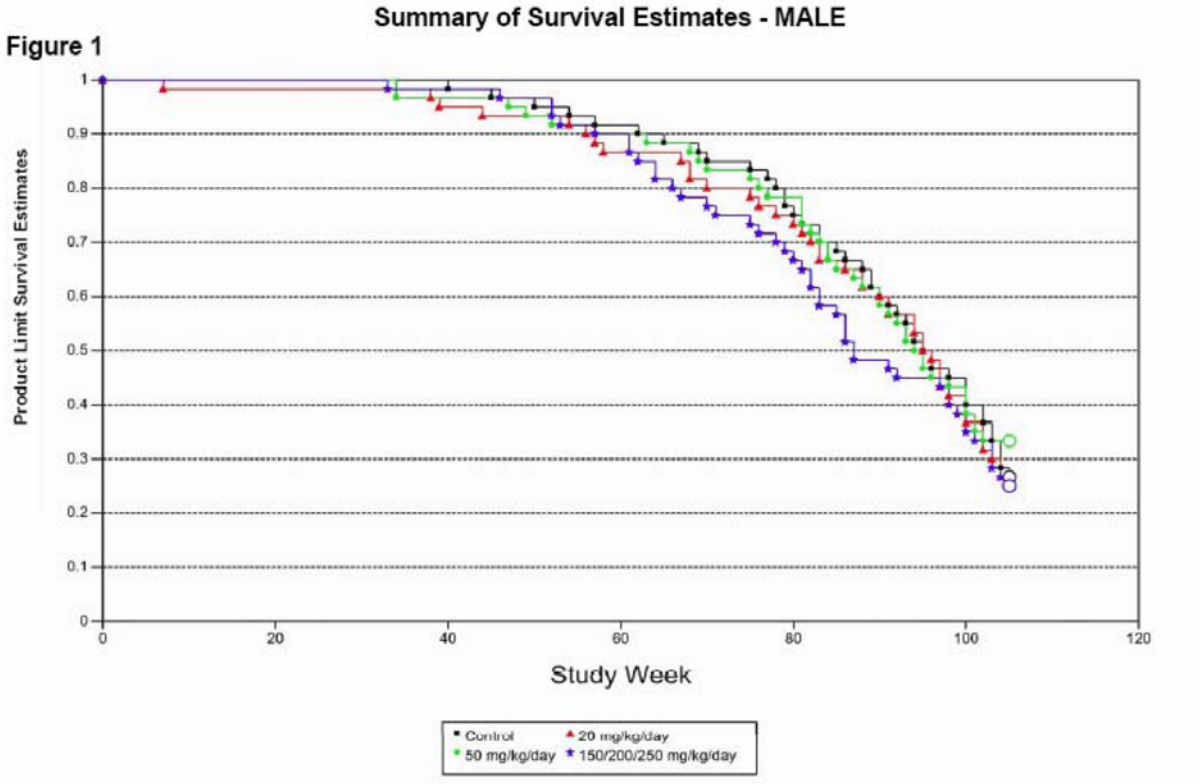


Figure 1

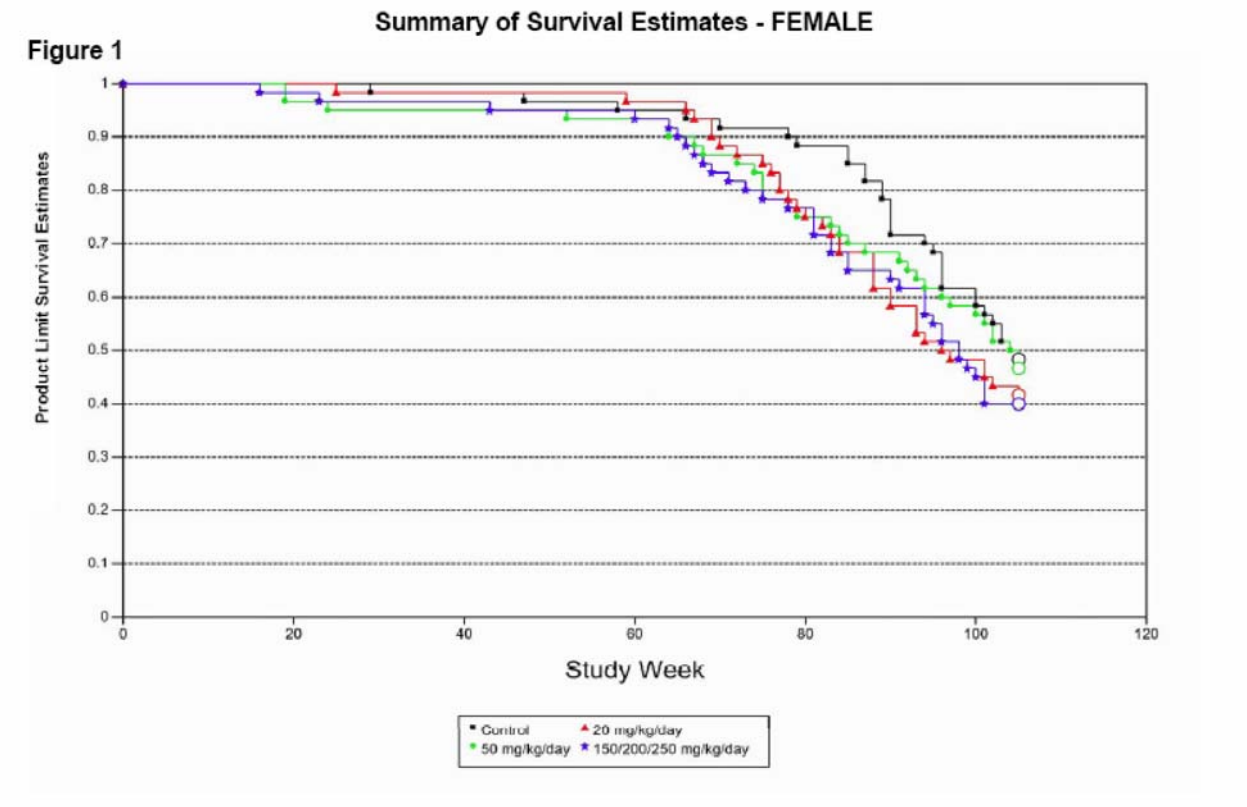


Table 2. Bodyweights in Study 1310-001: Rifaximin: A 2-year oral carcinogenicity study in rats

Body Weights, g						
	Males			Females		
Dose Level (mg/kg/day)	Pretest	Week 104	(%)	Pretest	Week 104	(%)
Vehicle Control	261.2	772.2	NA	192.1	532.0	NA
20	261.8	770.8	(-0.2)	192.2	480.1	(-9.8)
50	259.5	748.5	(-3.1)	191.5	532.0	(0)
150/200/250	262.1	759.0	(-1.7)	191.7	496.5	(-6.7)
NA – Not Applicable (%) – Percent difference from vehicle control at Week 104						

Figure 3 Mean Food Consumption Values – MALE

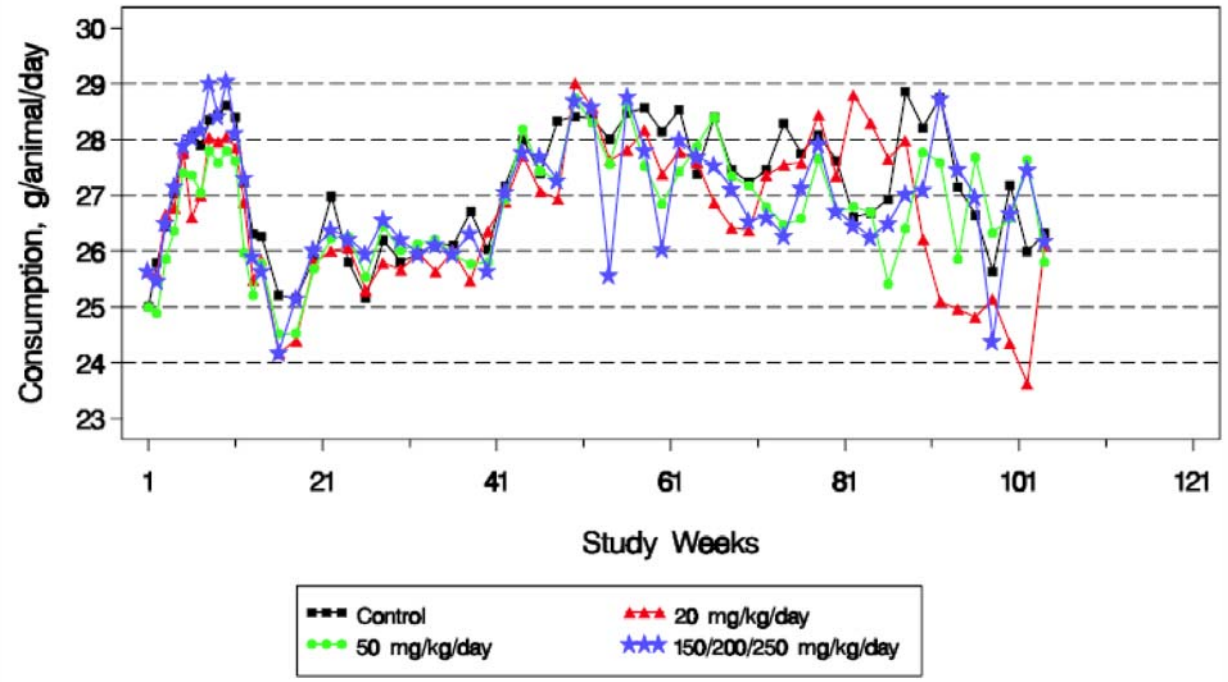


Figure 3A Mean Food Consumption Values – FEMALE

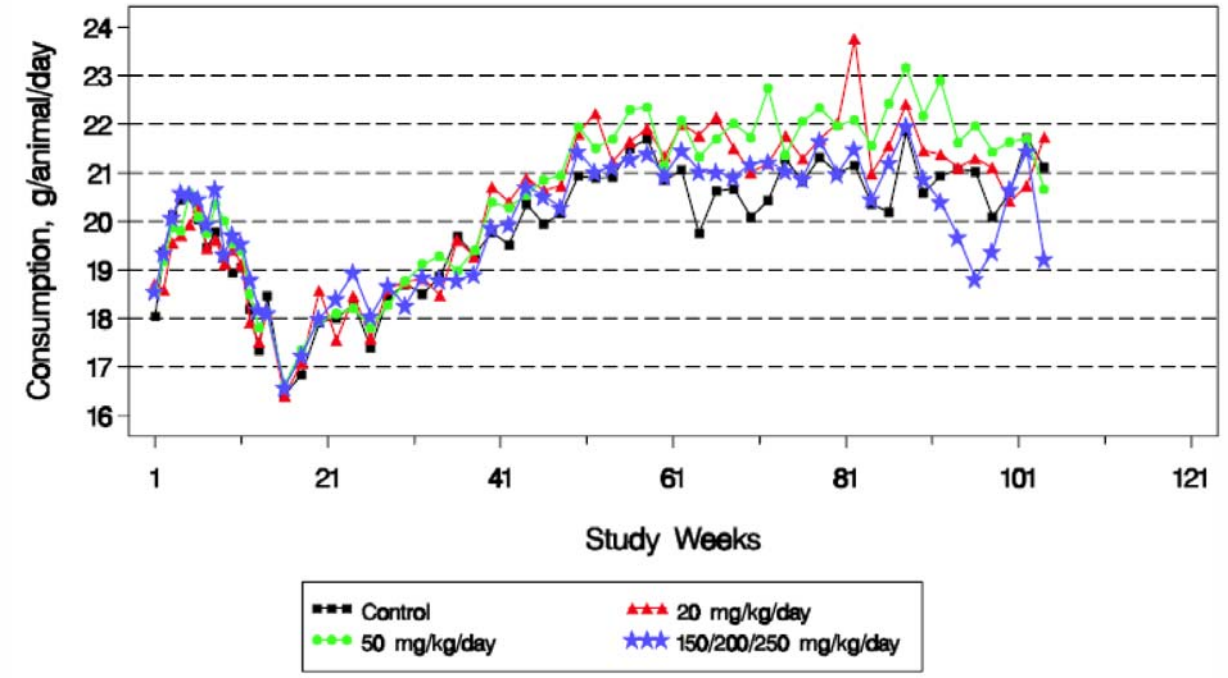


Table 3. Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons

Sex	Organ Name	Tumor Name	0 mg Cont N=60	20 mg Low N=60	50 mg Med N=60	150/200/250* mg High N=60	P-Value Dose Resp	C vs. L	C vs. M	C vs. H
MALE	HEART	SCHWANNOMA, MALIGNAN	0	0	1	3	0.0165*	.	0.4940	0.1071
	KIDNEYS	LIPOSARCOMA, MALIGNA	1	0	0	3	0.0436	0.4878	0.4940	0.2801
	PANCREAS	CARCINOMA, ISLET CEL	0	1	1	3	0.0358*	0.4878	0.4940	0.1027
	PITUITARY GLAND	ADENOMA, PARS DISTAL	34	45	40	30	0.9564	0.0319	0.2112	0.6409
FEMALE	ADRENAL GLANDS	ADENOMA, CORTICAL, B	4	8	6	12	0.0138	0.1295	0.3301	0.0146
	UTERUS WITH CER	POLYP, STROMAL, BENI	4	3	4	9	0.0155	0.4394	0.6066	0.0794

***At Week 28 the dose level was increased to 200 mg/kg/day, and at Week 39 the dose level was increased again to 250 mg/kg/day for the remainder of the study**

The incidences of malignant schwannoma in heart and islet cell carcinoma in pancreas in male rats were considered to have statistically significant positive dose response relationships. None of the pairwise comparisons were found to be statistically significant.

Table 4. Dosing Formulation evaluations

Dosing Formulation Analysis Sample Collection							
	Dose Level Sampled		Number of Samples per Concentration			Sample Volume	Intervals
Sample Type	(mg/kg/day)	Stratum	Collected	Analyzed	Backup	(mL)	(Week)
Homogeneity Analyses[a]	20, 150	Top	2	1	1	2.0	1, 24
		Middle	2	1	1	2.0	
		Bottom	2	1	1	2.0	
Homogeneity Analyses[a]	200	Top	2	1	1	2.0	28
		Middle	2	1	1	2.0	
		Bottom	2	1	1	2.0	
Homogeneity Analyses[a]	250	Top	2	1	1	2.0	39
		Middle	2	1	1	2.0	
		Bottom	2	1	1	2.0	
Concentration Analyses[a]	0, 20, 50, 150	Middle	1	1	0	2.0	1 to 4, 14, 27
Concentration Analyses[a]	200	Middle	1	1	0	2.0	28
Concentration Analyses[a]	0, 20, 50, 250	Middle	1	1	0	2.0	39[b], 40, 53, 66, 79, 92, 104
7[c] and 14[d] Day Stability Analysis	20, 50, 150	Middle	1	1	0	2.0	1
7[c] and 14[d] Day Stability Analysis	200	Middle	1	1	0	2.0	28
7[c] and 14[d, e] Day Stability Analysis	250	Middle	1	1	0	2.0	39

[a]The samples, including backup samples, were stored frozen at -20°C pending analysis or final disposition.
[b]250 mg/kg/day only sampled.
[c]Samples were stored at room temperature for 7 days, and then frozen at -20°C until analyzed.
[d]Samples were stored at room temperature for 14 days, and then frozen at -20°C until analyzed.
[e]Samples were not analyzed on Day 14 due to standards prepared with expired test article. A new preparation of standards was used for analysis of Day 14 stability samples on Day 16.

Table 5. Verification of Dosing Formulations

Concentration			
		Range of Average Calculated Concentrations	
Dose Level (mg/kg/day)	Nominal Concentration (mg/mL)	(mg/mL)	Average % Recovery[a]
Control	0.0000	0.0000 – (b) (4)	NA
20	2.0000	(b) (4)	
50	5.0000		
150	15.0000		
200	20.0000		
250	25.0000		

[a]Average % recovery was calculated from the nominal concentration.
[b]A positive result was observed from both Week 3 samples, and one Week 4 control sample. The positive result obtained was below the Lower Limit of Quantitation (LLOQ) indicated in the Analytical Method presented in Appendix B.
NA - Not Applicable

Table 6. Stability of Dosing Formulations

Stability								
Dose Level (mg/kg/day)	Initial Calculated Concentration (mg/mL)	Average Calculated Concentration (mg/mL) (Day)			Average % Recovery (Day)			
		7	14	16[f]	7	14	16[f]	
20 50 150 200 250[d]	[redacted]							(b) (4)

[a]Average % recovery is based on the average calculated sample concentration from Week 1 concentration analysis of dosing formulations.
[b]Data failed to meet the acceptance criterion for recovery (b) (4).
[c]Average % recovery is based on the average calculated sample concentration from Week 28 concentration analysis of dosing formulations.
[d]Data for the 250 mg/kg/day samples are the results of a reinjection due to a preparation error during the original analysis.
[e]Average % recovery is based on the average calculated sample concentration from Week 39 concentration analysis of dosing formulations.
[f]Samples were stored at room temperature for 14 days, and then frozen at -20°C until analyzed on Day 16 due to expired reference standards which needed to be re-prepared.
NA - Not Applicable

Table 7. Homogeneity of Dosing Formulations

Homogeneity				
Dose Level (mg/kg/day)	Nominal Concentration (mg/mL)	Average Calculated Concentration Range[b] (mg/mL)	Average % Recovery Range[a, b]	Range of RSD (%)[b]
20 150 200 250	2.0000 15.0000 20.0000 25.0000	[redacted]		(b) (4)

[a]The average % recovery range was calculated from the nominal concentration.
RSD - Relative Standard Deviation
[b]For clarification, range is presented as a maximum of two averages for the 20 and 150 mg/kg/day dose levels.

Addendum

On December 15, 2009, the Executive CAC evaluated the results of this rat two year carcinogenicity study and a twenty-six week carcinogenicity study in Tg.rasH2 mice. The Committee concurred that the malignant schwannomas in the heart of male rats were drug-related, based on a significant, positive dose-response trend and the incidence of this tumor being outside of the range of historical control data. The Committee also concurred that there were no drug-related neoplasms in the Tg.rasH2 mice. The following statement will be added to the XIFAXIN label:

(b) (4)

9 Reproductive and Developmental Toxicology

The following section contains summaries and excerpts from Dr. Steven Kunder's review of NDA 21,361 (April 22, 2004).

9.1 Fertility and Early Embryonic Development

In a fertility and reproductive toxicity study in male and female rats, rifaximin was administered orally at doses of 0, 25, 50 and 100 mg/kg/day. Dr. Kunder's review emphasizes that "this report was a sub-optimal translation from Italian of a pre-GLP study. It lacked figures, tables, and was generally poorly organized." No treatment-related changes were present in the clinical condition, body weights, or the fertility of F_0 males and F_0 females. Two 50 mg/kg/day females died due to gavage error, but all other animals survived until terminal sacrifice. There were no treatment-related histopathology changes in male reproductive organs. The mid-gestation maternal parameters (number of implants, resorptions, and live fetuses), and litter size at parturition were comparable among all F_0 groups. During the postnatal phase, the F_1 pups had comparable body weights and maturation (physical and neuromuscular development indices). The fertility of F_1 offspring was comparable among all groups. Based on the results, rifaximin at oral doses as high as 100 mg/kg/day had no adverse effects on general fertility in male and female rats, or on postnatal development and reproductive performance of the offspring.

In a preliminary study of effects on fertility and peri- and post-natal development in rats, rifaximin was administered orally at doses of 0, 150, and 300 mg/kg/day. There were no treatment-related changes in clinical observations. Decreased weight gain and food

consumption were generally present for the duration of the study in the 150 and 300 mg/kg/day groups. There were no meaningful changes in the length of estrus cycle, mating behavior, fertility, gestation length, litter size, and pup weights. No gross pathologic findings attributable to treatment were observed at study termination. There was a possible drug-related effect in one 300 mg/kg/day male. This male was euthanized on day 30 due to deteriorating clinical condition, which included pallor and bodyweight loss. After necropsy, a yellow discoloration of most of the internal organs was observed. This finding is in contradiction to the apparent minimal absorption seen in other studies and raises a question about the potential toxicity of rifaximin when its absorption is enhanced.

9.2 Embryonic Fetal Development

In a fertility and embryo-fetal developmental study in rats, rifaximin was administered orally at doses of 0, 50, 150, and 300 mg/kg/day. All rats survived for the duration of the study, and there were no meaningful treatment-related changes in the clinical observations. The 300 mg/kg/day females showed a decreased weight gain throughout the study period, but there were no changes in food consumption. There were no consistent treatment-related weight changes in reproductive organs of male rats. However, relative to controls, the 300 mg/kg/day group did show a slight decrease in reproductive organ weights during the later (post-pairing) phase. There were no meaningful changes in the length of estrus cycle, fertility, litter size, corpora lutea, implantations, resorptions, live litter size, or fetal weights, however placental weights in treated groups were lower than that of controls. An increase in the number of subcutaneous hemorrhages was noted in the 150 and 300 mg/kg/day groups. There was also an increased incidence of hemorrhages within the liver in the 300 mg/kg/day group. An increased incidence of incomplete ossification of cranial bones and pelvic bones were observed at doses of 150 and 300 mg/kg/day. The NOAEL for adult rats was 150 mg/kg/day, and NOAEL for fetuses was 50 mg/kg/day.

In an embryo-fetal developmental study in rabbits, rifaximin was administered orally at doses of 0, 62.5, 250, and 1000 mg/kg/day to pregnant rabbits from day 6 to day 19 of gestation. The plasma concentrations of rifaximin were measured predose, 1, 2, 4, 6, and 8 hours after dosing on gestation days 6 and 19. No treatment-related clinical changes or mortalities were present for the duration of the study. Four does (2 in the control group and 2 in the 250 mg/kg/day group) aborted, and one doe in the control group died prior to scheduled termination of the study from incidental causes. An initial weight loss (resulting in decreased weight gain) and a decrease in food consumption were seen during treatment in rifaximin-treated rabbits. Food consumption was reduced 20% to 25% in all groups during the first week of treatment. There were several malformations noted in fetuses, which included an increased incidence of 20 thoracolumbar vertebrae (all treated groups), subdural hemorrhage (250 and 1000 mg/kg/day), partially open eye (1000 mg/kg/day), one fetus with small eyes, retinal irregularities, cleft palate, brachygnathia, small displaced kidneys, sternebral irregularities, large atrium, interventricular septal defect (250 mg/kg). The maternal NOAEL was 62.5 mg/kg/day. The fetal NOAEL was < 62.5 mg/kg/day, due to the fetal

effects at all doses,. Systemic exposure of rifaximin was lower than the limit of detection in the 62.5 mg/kg/day group, and was minimal in the 250 mg/kg/day (AUC_{0-8} = 6.63-8.25 ng·h/mL) and 1000 mg/kg/day groups (AUC_{0-8} = 10.95-16.97 ng·h/mL).

9.3 Prenatal and Postnatal Development

In a combined embryo-fetal, peri-/post-natal developmental study in rats, rifaximin was administered orally at doses of 0, 50, and 100 mg/kg/day to pregnant rats from day 5 through day 18 of gestation. A subset of dams were sacrificed on gestation day 21, and the fetuses were removed and examined. Dr. Kunder's review emphasizes that "this report was a sub-optimal translation from Italian of a pre-GLP study" and "the lack of details devalues the study itself." There were no treatment-related changes in clinical observations or in body weights. This study also included evaluations of hematologic and blood lipid parameters at necropsy; there were no changes in these parameters. The maternal parameters, litter size, and the fetal weights were comparable among all groups. External, skeletal, and visceral examinations did not reveal any teratogenic effects attributable to rifaximin. Based on the results, rifaximin at oral doses as high as 100 mg/kg/day in pregnant rats had no adverse effects on embyo-fetal or postnatal development of the offspring. The maternal and fetal NOAEL was 100 mg/kg/day.

In a combined embryo-fetal, peri-/post-natal toxicity study in rabbits, rifaximin was administered orally at doses of 0, 50, and 100 mg/kg/day to pregnant rabbits from day 6 through day 18 of gestation. Rabbits were sacrificed on gestation day 28, and the number of resorptions, implantations, fetuses (live and dead), and fetal weights were recorded. Dr. Kunder's review emphasizes that "this report was a sub-optimal translation from Italian of a pre-GLP study" and "the lack of details devalues the study itself." There were no treatment-related changes in clinical observations or in body weights. This study also included evaluations of hematologic and clinical chemistry parameters at necropsy; there were no changes in these parameters. The maternal parameters and fetal weights were comparable among all groups. External, skeletal, and visceral examinations did not reveal any teratogenic effects attributable to rifaximin. Based on the results, rifaximin was concluded to be not teratogenic when administered orally to pregnant rabbits during organogenesis at doses as high as 100 mg/kg/day. The maternal and fetal NOAEL were 100 mg/kg/day. The absence of effects on fetal development is inconsistent with results observed in the embryo-fetal developmental study in rabbits, described above.

In a pre-/post-natal developmental study in rats, rifaximin was administered orally at doses of 0, 50, 150, and 300 mg/kg/day to pregnant rats from day 6 of gestation to day 20 of lactation. One F_1 dam (300 mg/kg/day) died during the last week of the study following a significant decrease in weight. There were no meaningful changes in clinical observations or food consumption in treated F_0 females. In the 150 and 300 mg/kg/day groups, dams exhibited decreased weight gain and decreased food consumption, relative to controls. The litter size, duration and nature of parturition, and pup weights were comparable among all F_0 groups. There was no effect on maturation indices or pre-weaning physical development. During the postnatal phase, the F_1 pups had comparable body weights and maturation (physical, auditory and visual development,

neuromuscular development, and learning indices). The fertility of F_1 offspring including F_2 pup litter size and weights were comparable among all groups. Therefore, peri- and post-natal development and reproductive performance in the F_1 generation appeared to be unaffected by F_0 maternal treatment. In the F_0 dams, the NOAEL was 50 mg/kg/day.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Liver was identified as a target organ of toxicity in rats and dogs, in the original set of toxicity studies (oral) on rifaximin (pre-GLP studies conducted by a different Sponsor). These studies did not include toxicokinetic measurements. The current Sponsor conducted GLP-compliant oral toxicity studies in rats (26 weeks) and dogs (39 weeks). No effects in liver were observed in the GLP-compliant toxicity studies, despite the use of doses that exceeded the hepatotoxic dose levels in the pre-GLP studies. The GLP studies included toxicokinetic data, which showed the presence of low levels of rifaximin in plasma. There is no apparent reason for the discrepancy between the pre-GLP and GLP studies with respect to hepatic effects. One possible explanation would be higher drug absorption in the pre-GLP studies, due to a difference in the drug formulation.

Systemic exposure to rifaximin is minimal in animals and humans following oral administration. However, dogs exhibited a 12% bioavailability for total radioactivity using [^{14}C]rifaximin administration, as compared to 2% bioavailability in rats. The metabolism of rifaximin was not studied. Toxicokinetic data from chronic studies were limited, consisting of data from only one chronic (GLP-compliant 26-week) rat study and one chronic (GLP-compliant 39-week) dog study. Minimal signs of toxicity were noted in these studies, which showed a range of AUC values (42 to 127 ng·hr/ml) that was generally lower than the mean AUC observed in cirrhotic patients (130 ± 78 ng·hr/ml, with a range of 28 to 412 ng·hr/ml). In addition, intravenous toxicity studies were not performed. Therefore, the dataset from animal toxicity studies provides only limited information about the potential for systemic effects, and does not provide assurance of safety for the use of rifaximin in cirrhotic patients, where increased systemic exposure is expected.

The increased systemic exposure in cirrhotic patients is a cause for concern, given the potential for rifaximin-induced liver injury. Furthermore, it will be difficult to monitor for drug-induced liver injury in cirrhotic patients. In order to address the safety concern about higher AUCs in cirrhotic patients, the Sponsor must conduct a chronic oral toxicology study that evaluates plasma AUC exposure in animals that is comparable to the highest plasma AUCs observed in cirrhotic patients (approximately 400 ng·hr/ml). These AUC values must be achieved and maintained throughout the duration of the chronic toxicity study. In the event that sufficiently high AUC levels cannot be achieved

from oral dosing, alternative routes of administration may be used. The repeat-dose toxicity study that is needed to address the safety concern of increased AUC values in cirrhotic patients may be conducted as a post-marketing study.

12 Appendix/Attachments

APPENDIX-I: "NDA 21-361 Pharmacology/Toxicology Labeling Supplement Review (SLR-009)" by Steve Hundley (June 16, 2009)

NDA 21-361 Pharmacology/Toxicology Labelling Supplement Review

NDA 21-361
SLR - 009; Labelling Supplement - Revision Request
Drug: Xifaxan® (Rifaximin) Tablets
Applicant: Salix Pharmaceuticals, Inc.
1700 Perimeter Park Drive
Morrisville, NC 27560
919 - 862-1000

Submission Date: 2/9/09
Review Date: 6/9/09

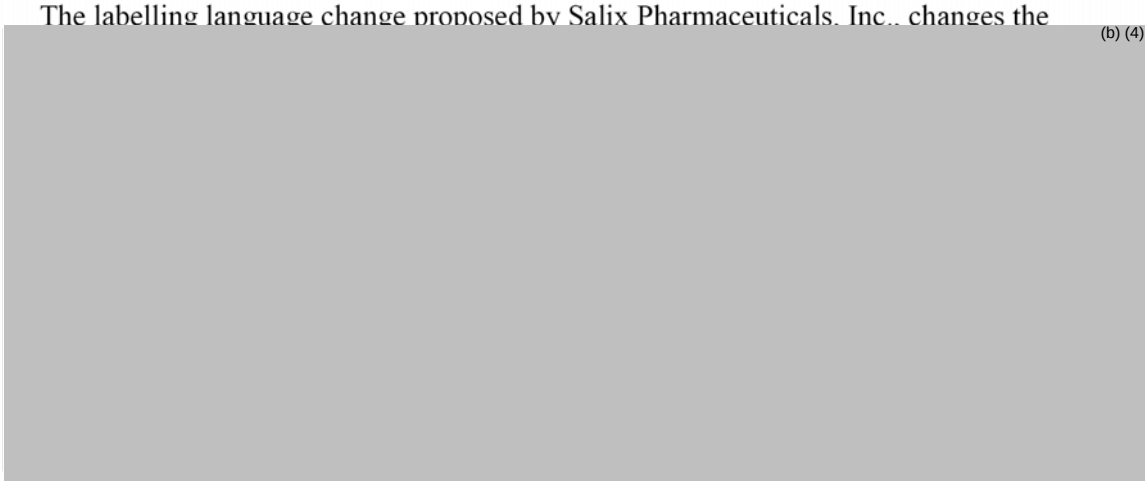
Background

Xifaxan® was approved on 5/25/04 for the treatment of traveler's diarrhea. The applicant requests that the Pregnancy Category of the current product label be revised. The Pharmacology/Toxicology Review of NDA 21-361 issued on 2/4/03, recommended the current labelling language as excerpted from the product label.

> *Pregnancy - Teratogenic Effects (Pregnancy Category C)*
>
> *Pregnancy*
>
> *Pregnancy category C: Rifaximin was teratogenic in rats at doses of 150 to 300 mg/kg (approximately 2.5 to 5 times the clinical dose, adjusted for body surface area) and in rabbits at doses of 62.5 to 1000 mg/kg (approximately 2 to 33 times the clinical dose, adjusted for body surface area). These effects include cleft palate, agnatha, jaw shorteninig, hemorrhage, eye partially open, small eyes, brachygnathia, incomplete ossification, and increased thoracolumbar vertebrea. There are no adequate and well controlled studies in child bearing women. Xifaxan® tablets should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus.*

The labelling language change proposed by Salix Pharmaceuticals, Inc., changes the

(b) (4)



(b) (4)

The applicant submitted several reproductive toxicity studies in rats and rabbits. These studies were previously submitted to the NDA and were critically examined by the Pharmacology/Toxicology Reviewer. The Pharmacology/Toxicology review and evaluation of these studies is contained in the review of NDA 21-361 issued on 2/4/03. The sponsor also submitted a reevaluation of the pivotal studies prepared by (b) (4) (b) (4) entitled (b) (4) Overview of Embyo-Fetal Toxicity Studies in the Rat and Rabbit Dosed with Rifaximin."

Reproductive Toxicity Study Titles

Study # N2266, Rifaximin: Combined Study of Effects on Fertility and Embryo-Fetal Toxicity in CD Rats by Oral Gavage Administration.

Study # N2154, Rifaximin: Preliminary Embryo-Foetal Toxicity Study in the Rabbit by Oral Gavage Administration.

Study # N2155, Rifaximin: Study of Effects on Embryo-Fetal Toxicity in the Rabbit by Oral Gavage Administration.

Study # N2008, L105 (Rifaximine) Fetal, Perinatal, and Postnatal Toxicity Test.

Study # N2015, L105 (Rifaximine) Study on the Fertility and Reproduction in the Rat.

Study # N2174, Rifaximin: Preliminary Study of Effects on Fertility and Peri- and Post-Natal Development in CD Rats by Oral Gavage Administration.

Study # N2265, Rifaximin: Study of Effects on Pre- and Post-Natal Development in CD Rats by Oral Gavage Administration.

All of these study were previously reviewed (Pharmacology/Toxicology Review of NDA 21-361, Review Completion Date: 2/4/03).

Review and Evaluation

The Pharmacology/Toxicology Review of NDA 21-361 relied on results from the definitive embryo-fetal developmental toxicity studies with rats and rabbits to provide the basis for the Pregnancy Category C designation (Study # N2266, Rifaximin: Combined Study of Effects on Fertility and Embryo-Fetal Toxicity in CD Rats by Oral Gavage

Administration and Study # N2155, Rifaximin: Study of Effects on Embryo-Fetal Toxicity in the Rabbit by Oral Gavage Administration). The major malformations observed in these two studies are presented in the following outline.

Fetal Malformations (New Zealand White Rabbits)

62.5 mg/kg Dose (180 fetuses examined)

1 fetus: Cebocephaly; Posterior Cardinal vein persistence
1 fetus: Posterior Cardinal vein persistence
1 fetus: Sternebral irregularities (3^{rd} to 6^{th} fused, 1^{st} and 6^{th} cleft, ventral curve)

250 mg/kg Dose (172 fetuses examined)

1 fetus: Cleft palate (soft tissue protrusion)
1 fetus: Lumbar Scoliosis (hemivertebra, 5^{th} lumbar)
1 fetus: Cleft palate; Sternebral irregularities (fused 1^{st} to 6^{th} , wide 2^{nd} to 5^{th}); Small eye, vitreous humour absent, Retinal irregularities (folded and detached); Brachygnathia; Interventricular septal defect; Dilated pulmonary trunk; Small displaced kidneys; Large Atrium

1,000 mg/kg Dose (164 fetuses examined)

2 fetuses: Posterior Cardinal vein persistence
1 fetus: Partially open eye

Fetal Malformations (Sprague-Dawley Rats)

50 mg/kg Dose (330 fetuses examined)

1 fetus: Folded retina
1 fetus: Inferior vena cava duplicated (Thoracic region)
1 fetus: Kinked irregularly ossified ribs (3^{rd} to 13^{th} ,Marked); Bent Scapula & Clavicle

150 mg/kg Dose (342 fetuses examined)

1 fetus*: Cranioschisis; Ablepharia; protruding tongue;
1 fetus*: Cranioschisis; Ablepharia; protruding tongue; Cleft palate & lip
1 fetus*: Cranioraschischisis; Ablepharia; protruding tongue; short maxillae
1 fetus*: Cranioraschischisis; Ablepharia; protruding tongue; short maxillae Lordosis (cervical) & Kyphosis (Thoracolumbar); Bilateral forelimb Flexure
1 fetues*: Vertebral column termination (Sacral region); Short thread like tail

* Fetuses were from the same litter.

300 mg/kg Dose (314 fetuses examined)

1 fetus: Anophthalmia
1 fetus: Retinal irregularities; Small eye

None of the previously listed observations for New Zealand white rabbit and Sprague-Dawley rat fetuses were observed in fetuses from the respective zero-level vehicle control groups. These malformations occurred at low incidence rates and in the absence of an apparent dose response. However, most of the malformations are considered major and rare with historical control rates of 0.01 to 0.04 percent based upon the accumulation of historical control data by (b) (4) (internet web site) for Sprague-Dawley CD rats (55 studies from 1996 - 2007 with approximately 17,000 fetuses) and New Zealand white rabbits (62 studies from 1983 - 2007 with approximately 9,000 fetuses).

No dose response for the observed malformations in rats and rabbits was apparent. However, rifaximin is poorly absorbed following oral administration and toxicokinetic data from the original Pharmacology/Toxicology NDA review (2/4/03) indicated overlapping plasma AUCs for the mid and high oral dose levels to rats and rabbits as indicated below.

Rats (sexes combined; Day 1 of a six month toxicity study)

150 mg/kg dose AUC = 92 ng · hr/ml (group average)
300 mg/kg dose AUC = 127 ng · hr/ml (group average)

Pregnant Female Rabbits[a]

Gestation Day 6		Gestation Day 19	
AUCs (Descending) ng · hr/ml	Dose Level mg/kg	AUCs (Descending) ng · hr/ml	Dose Level mg/kg
22.8	1,000	22.8	1,000
9.0	**250**	18.2	1,000
7.7	1,000	15.5	1,000
7.6	1,000	12.8	**250**
6.3	**250**	11.4	1,000
5.6	1,000	7.7	250
4.6	250	7.3	250
---	250	5.1	250

[a]Toxicokinetic determinations were made using four pregnant female rabbits at each dose (Study # N2155).

The toxicokinetic data from the rats (males and non-pregnant females from a six-month repeat dose toxicity study) and pregnant female rabbits indicated an overlapping of systemic exposure to rifaximin with increased administered dose. These data may be used to explain the absence of a dose response in the observation of external malformations in developing fetuses.

The sponsor submitted (b) (4) laboratory historical control data for variations and anomalies in CD rat and New Zealand White rabbit fetuses. The following fetal effects listed in the current label need to be removed based upon the zero-level vehicle control

rates: hemorrhage, incomplete ossification, and increased thoracolumbar veterbra. The malformation of agnatha needs to be removed because it was only observed in the preliminary rabbit embryo/fetal develomental toxiciy study and not in the pivotal study with 20 pregnant dams at each exposure level.

The sponsor indicated that malformations observed in fetal rabbits should be discounted because bactericidal activity of rifaximin alters the gut microflora. The maternal toxicity observed in the pivotal study was limited to reduction in body weight gain at each rifaximin dose level. Clinical and gross pathological effects were not observed. No differences were observed between the zero-level vehicle control and rifaximin dose levels in the number of pregnant females that aborted. In addition, maternal/embryo-fetal adverse effects were not observed in the following reproduction parameters: number of corpora lutea, number of implantation sites, viable fetuses, early/late resorptions, percent implantation loss, fetal weights, and placental weights. The malformations observed in fetal rabbits need to be included because compound-related effects were not observed in the previously listed maternal/embryo-fetal reproduction parameters.

The absence of a dose response in the observed rabbit fetal malformations was possibly due to the absence of substantive systemic rifaximin exposure differences between the 250 and 1,000 mg/kg dose levels. The following malformations need to be retained or included in the revised label: cleft palate, lumbar scoliosis, eye malformations, brachygnathia, and possibly interventricular septal defect, and large atrium. Posterior cardinal vein perisistence and sternebral irreglarities were observed at the 62.5 mg/kg dose level but should not be included because the toxicokinetic phase of the study did not result in detectable levels of rifaximin at this dose level.

The sponsor did not address the issue of malformations observed in fetal rats. The Pharmacology/Toxicology Review of NDA 21-361 and the approved product label included these malformations as compound related. The malformations observed at the 150 mg/kg dose level were observed in five fetuses from the same litter. The relevance of multiple observations isolated in one litter is questionable and are generally discounted as being compound-related. Therefore, the rat fetal malformations observed at the 150 mg/kg dose level will not be included in the product label. The eye malformations (anophthalmia, retinal irregularities, and small eyes) observed at the maternally toxic dose of 300 mg/kg will be included. Inclusion of eye malformations can be justified due to the absence of maternal and embryo-fetal effects for number of corpora lutea, number of implantations, viable fetuses, early/late resorptions, percent implantation loss, fetal weights, and placental weights, and due to the presence of eye malformations in fetal rabbits.

The correlation between dose levels in the rat and rabbit embryo-fetal developmental toxicity studies and therapeutic dose levels to patients with diarrhea needs to be adjusted to reflect the systemic exposure to rifaximin as expressed by plasma AUC values. The approximate rifaximin AUC in human patients is 8 ng · hr/ml based upon pharmacokinetic data listed in the current product label. The rat (300 mg/kg)/human

AUC ratio is approximately 16. The rabbit/human AUC ratio is approximately 1and 2 for the 250 and 1,000 mg/kg dose levels, respectively.

Recommended Pregnancy Category Labelling Language

The Pharmacology/Toxicology Reviewer recommends the following labelling language for the Xifaxan® (rifaximin) tablet product label based upon information provided by the applicant and a reexamination of the embryo-fetal developmental toxicity data from the original NDA 21-361 submission.

Pregnancy - Teratogenic Effects (Pregnancy Category C)

Pregnancy

Pregnancy category C: There are no adequate and well controlled studies in child bearing women. Rifaximin resulted in eye malformations in rat and rabbit fetuses at dose levels that exhibited maternal effects (reduced body weight gain). Additional malformations were observed in fetal rabbits that included cleft palate, lumbar scoliosis, brachygnathia, interventricular septal defect, and large atrium. The malformations were observed at the high dose level to pregnant female rats and the mid and high dose levels to female rabbits that were, respectively, 16-fold and 1- or 2-fold the therapeutic dose to diarrhetic patients based upon plasma AUC comparisons. Post-natal developmental effects were not observed in rat pups from pregnant/lactating female rats dosed during gestion to Day 20 post-partum at the highest dose that was approximately 16-fold greater than the human therapeutic dose (based upon AUC comparison). Xifaxan® tablets should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus.

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Concurrence:

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/s/

Steve Hundley
6/9/2009 11:57:16 AM
PHARMACOLOGIST

William Taylor
6/16/2009 07:12:46 AM
PHARMACOLOGIST

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22554	ORIG-1	SALIX PHARMACEUTICALS INC	XIFAXAN

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/s/

NIRAJ R MEHTA
03/15/2010

DAVID B JOSEPH
03/16/2010
I concur with Dr. Mehta's recommendations. I have no additional comments.