CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-908

PHARMACOLOGY REVIEW(S)

Addendum To December 23, 2005 Pharmacologist's Review of NDA 21-908 (AMITIZA).

Dr. Jacob's draft comments, dated 1/12/06 are addressed in the following sections.

I. The pharm/tox review:

A. The review was finalized before the executive CAC Committee meeting. Thus, the executive summary of the review was not the exact reflection of the Executive CAC Committee recommendations.

The following statement should replace para 4 of page 5 of the 'Executive Summary' section of the Pharmacology Review.

'The carcinogenic potential of lubiprostone (RU-0211) was assessed in a 104-week oral carcinogenicity study in mice and a 104-week oral carcinogenicity study in rats. In the 2-year carcinogenicity study in mice, lubiprostone doses of 25, 75, 200 and 500 µg/kg/day (approximately 2, 6, 17 and 42 times the human dose, respectively, based on body surface area) were used. In the 2-rear carcinogenicity study in rats, lubiprostone doses of 20, 100 and 400 µg/kg/day (approximately 3, 17 and 68 times the human dose, respectively, based on body surface area) were used. In the mouse carcinogenicity study, there was no significant increase in any tumor incidences. In male rats, there was a significant increase in the incidence of interstitial adenoma of the testes at the 400 µg/kg/day dose (p=0.0035). In female rats, treatment with lubiprostone produced hepatocellular adenoma at the 400 µg/kg/day dose (p=0.0259)'.
It is not appropriate to use 'exposure ratios' for the following reasons: The parent compound was not detectable in humans even after administration of a dose 3-fold higher than the recommended clinical dose (Dr. Alfayoumi's Review, 12/28/2005). In carcinogenicity studies, the toxicokinetics of the parent compound was determined, but metabolites were not included. Therefore, the ratios based on surface area doses should be retained.

B. The Pharmacology recommendations on labeling are included from page 193 of the review.

II The preclinical portions of labeling:

A. Carcinogenesis section:

As recommended by Dr. Jacobs, the 'incidences' is changed to the 'incidence', and the 400 µg/kg dose is included for incidences of interstitial testicular and hepatocellular tumors. As mentioned above, the exposure levels in rats could not be compared with that in humans, because the parent compound was not detectable in humans. The high dose (400 µg/kg) in rats was 68 times higher than the clinical dose, based on body surface area (exposure levels were not 68-fold of that in humans).

B. Teratogenicity section:

The sentence stating [redacted] is replaced with the standard pregnancy category C statement. Because pup viability and reduced pup weight were observed only at a maternal toxic dose of 1.0 mg/kg (mortalities during parturition, abnormal clinical signs, and 28.4% to 34.6% suppression of body weight gains), these findings do not merit inclusion in the labeling.

C. Nursing Mothers

Irregardless of the dose ratios, the occurrence of tumors in each sex merits mentioning in the label which is in accord with wordings in 21CFR, 201.57 for "Nursing Mothers". As mentioned above, the 68-fold was the dosage level, not the exposure levels. We could not compare the exposure levels between rats and humans.

Sushanta Chakder, Ph. D. Date

Pharmacologist, HFD-180

Comments:

Jasti B. Choudary, Ph.D., B. V. Sc. Date

Supervisory Pharmacologist, HFD-180

cc.

NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Chakder

HFD-180/Dr. Choudary

OND IO/Dr Jacobs

R/D Init.: J. Choudary 1/13/06

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Sushanta Chakder
1/13/2006 11:35:17 AM
PHARMACOLOGIST

Jasti Choudary
1/13/2006 11:48:42 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-908**
SERIAL NUMBER:	**000/001/004**
DATE RECEIVED BY CENTER:	March 31, 2005/June 15, 2005/September 15, 2005
PRODUCT:	**RU-0211 (Lubiprostone) Capsules**
INTENDED CLINICAL POPULATION:	**Adult subjects with chronic constipation.**
SPONSOR:	**Sucampo Pharmaceuticals, Inc., Bethesda, MD.**
DOCUMENTS REVIEWED:	**Electronic submission of the NDA**
REVIEW DIVISION:	**Division of Gastroenterology Products (HFD-180)**
PHARM/TOX REVIEWER:	**Sushanta Chakder, Ph.D.**
PHARM/TOX SUPERVISOR:	**Jasti B. Choudary, B.V. Sc., Ph.D.**
DIVISION DIRECTOR:	**Brian Harvey, M.D., Ph.D.**
PROJECT MANAGER:	**Tanya Clayton, B.S.**

Date of review submission to Division File System (DFS): **December 23, 2005**

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APPEARS THIS WAY
ON ORIGINAL

EXECUTIVE SUMMARY

I. Recommendations

A. **Recommendation on approvability:** The sponsor conducted adequate preclinical studies with RU-0211 to determine the safety of the drug. Thus, from a preclinical standpoint, the NDA application is approvable. However, RU-0211 caused dose-dependent abortions in guinea pigs, when administered to the pregnant animals. So, the drug should not be used by pregnant women, and should have restricted use in women with child-bearing potential.

B. **Recommendation for nonclinical studies:** None

C. **Recommendations on labeling:** Included in the labeling section of the review.

II. Summary of nonclinical findings

A. Pharmacology:

RU-0211 is a prostaglandin E1 analog. It is a specific activator of CIC-2 chloride channels, which is involved in the secretion of fluids in the gastrointestinal tract. In human intestinal epithelial (T_{84}) cells, it caused a dose-dependent increase in chloride current. RU-0211 caused a dose-dependent augmentation of acetylcholine-induced contractions of isolated rat ileum. RU-0211 caused a dose-dependent increase in intestinal fluid secretion in rats. It had no effects on the serum levels of Na^+, K^+ and Cl^- in rats at oral doses up to 100 µg/kg. The main metabolite of RU-0211, 15-hydroxy-RU-0211 (M3) also caused a dose-dependent increase in the intestinal fluid secretion in rats, with a potency similar to that of the parent compound. RU-0211 at oral doses of 1 to 100 µg/kg, caused an increase in intestinal transit in morphine-treated mice without affecting the analgesic effects of morphine. It had no effects on the respiration rate, heart rate or ECG parameters of anesthetized dogs at intraduodenal doses up to 1000 µg/kg. It had no effect on the central nervous system of rats at oral doses up to 1000 µg/kg. RU-0211 did not cause a prolongation of action potentials in canine isolated canine cardiac Purkinje fibers.

B. ADME:

RU-0211 was rapidly absorbed in rats and dogs following oral administration. Following oral administration of single doses of 25, 50 and 100 µg/kg doses to rats, the maximum plasma concentrations were reached in 0.5 to 0.75 hr, and the terminal elimination half-lives ($t_{1/2}$) ranged from 2 to 6 hours. In mice, following oral administration of 25 and 50 µg/kg doses, the T_{max} was 10 to 15 minutes, and the $t_{1/2}$ was about 3 hours. In contrast to rats, in dogs the T_{max} for the total radioactivity was 3 hours following oral administration, and the $t_{1/2}$ was longer than in rats. In rats, there were no signs of accumulation of the drug in any specific tissue. The plasma protein binding of RU-0211 in rats (92 to 95%), dogs (96%) and humans (94-95%) was high. In rats, dogs and mouse, RU-0211 was metabolized rapidly, and several metabolic peaks were detected in the plasma samples. Among them, 15-hydroxy-RU-0211 was the least polar metabolite, and was found be biologically active, with a potency similar to the parent compound. No other metabolite showed the pharmacological activity. In *in vitro* studies with cells expressing different CYP enzymes, microsomal CYP enzymes had no major role in the metabolism of RU-0211. The parent compound was detected in plasma samples for up to 2 hours after administration. In rats, mice, dogs and monkeys, renal excretion was the predominant pathway for elimination of the drug, except following oral administration in rats.

C. Toxicology:

Single and repeat dose toxicology studies with RU-0211 were conducted in rats, mice and dogs. In the acute toxicity study in rats, the minimal lethal dose was 60 mg/kg in males and 30 mg/kg in females. The clinical signs observed in rats included decreased locomotor activity, lacrimation, loose stool and dyspnea. In dogs, single oral doses up to 40 mg/kg was non-lethal, and decreased locomotor activity, loose stool/diarrhea, vomiting, lacrimation, salivation and pale buccal mucosa were observed in males and females.

In repeat dose oral toxicity study in rats and mice and dogs, loose stool or diarrhea was observed in all species, which is related to the pharmacological actions of the drug. Proliferation of the epithelial basal cells in the limiting ridge of the stomach was observed in rats and mice. In a 2-week oral toxicity study in rats, fibrous osteodystrophy of the femur and sternum and decreased cellularity of the bone marrow were observed in both males and females at a dose of 5 mg/kg. However, no effect on the bone or bone marrow was observed in the 4-week and 6-month toxicity studies in rats. Hyperplasia of the zona glomerulosa of the adrenal gland was observed in rats, mice and dogs. In the chronic 39-week oral toxicity study in dogs, atrophy of the seminiferous tubule was observed in males and pyelitis in the kidneys was observed in males and females at a dose of 0.05 mg/kg/day. However, in reproductive toxicology studies, RU-0211 had no effects on the reproductive function of male rats at doses up to 1.0 mg/kg.

RU-0211 was not genotoxic in a battery of genotoxicity assay, including the Ames test, the *in vitro* chromosome aberration assay in Chinese hamster liver cells, the *in vitro* mouse lymphoma cell forward gene mutation assay and the *in vivo* mouse bone marrow micronucleus assay.

The carcinogenic potential of RU-0211 was assessed in a 104-week oral carcinogenicity study in mice and a 104-week oral carcinogenicity study in rats. In female mice, there was an increase incidence for Harderian gland carcinoma at the high dose (500 μg/kg/day).
Male rats receiving RU-0211 had higher incidences of squamous cell papilloma in nonglandular stomach. The combined incidences of squamous cell papilloma and carcinoma in male rats were also higher than that in control. The incidences of histiocytic sarcoma and benign interstitial cell tumor of the testes were significantly higher in male rats receiving the 400 μg/kg dose. In female rats, treatment with RU-0211 produced hepatocellular adenoma at 400 μg/kg.

In the oral Segment I fertility and general reproductive performance study with RU-0211 in rats, doses up to 0.2 mg/kg/day did not produce any effects on the fertility and reproductive performance of male and female animals. There was a decrease in the number of implantation sites and live embryos at a dose of 1.0 mg/kg/day. RU-0211 was not teratogenic in rats at oral doses up to 2.0 mg/kg/day. It was not teratogenic in rabbits at oral doses up to 0.10 mg/kg/day. In the Segment III pre- and post-natal developmental toxicity study with RU-0211 in rats, viabilities of pups and mean pup weights from dams receiving the 1.0 mg/kg/day dose were lower than that of controls.

The abortifacient potential of RU-0211 was examined in specific guinea pig and Rhesus monkey models following oral administration. Treatment with RU-0211 was associated with a dose-dependent abortion in guinea pigs. The dose selection for the monkey abortifacient study was based on NOEL in rats, and was not appropriate.

D. **Nonclinical safety issues relevant to clinical use**

RU-0211 was abortifacient in guinea pigs when administered to pregnant animals. Thus, it may endanger pregnancy when administered to pregnant women. So, the drug should not be taken by pregnant women. If a women is or becomes pregnant while taking this drug, the drug should be discontinued immediately and the patient be informed of the potential harm

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-908
Review number: 01
Sequence number/date/type of submission: 000/March 31, 2005/Original; 001/June 15, 2005; 004/September 15, 2005.
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Sucampo Pharmaceuticals, Inc., Bethesda, MD
Manufacturer for drug substance: R-Tech Ueno, Ltd., Sanda, Hyogo 669-1339, Japan
Reviewer name: Sushanta Chakder, Ph.D.
Division name: Division of Gastroenterology Products
HFD #: 180
Review completion date: 12/12/05

Drug:

Trade name:

Generic name: Lubiprostone

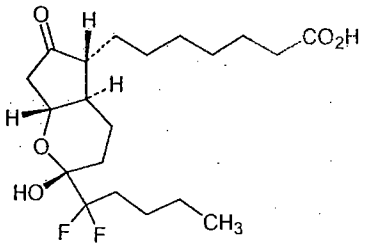
Code name: RU-0211/SPI-0211

Chemical name: (-)-7-[(2R, 4aR, 5R, 7aR)-2-(1, 1-difluoropentyl)-2-hydroxy-6-oxaoctahydrocyclopenta[b]pyran-5-yl] heptanoic acid.

CAS registry number: 136790-76-6, 333963-40-9

Molecular formula/molecular weight: $C_{20}H_{32}F_2O_5$/390.46

Structure:



Relevant INDs/NDAs/DMFs:
IND 59,623; RU-0211, for treatment of chronic constipation; Sucampo Pharmaceuticals Inc., Bethesda, MD.

Drug class: Prostaglandin analog
Intended clinical population: Adult subjects with chronic idiopathic constipation

Clinical formulation: Each soft gelatin capsule contains 24 µg RU-0211. in medium chain fatty acid triglyceride MCT)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

STUDY	STUDY #	LOT #	TESTING LAB	PAGE # OF REVIEW
Pharmacology				12
Absorption				
Pharmacokinetics of RU-0211 in rats following oral and i.v. administration	--	--	--	33
Pharmacokinetics of RU-0211 after single oral administration to male and female rats #	EIH116	--	--	37
Pharmacokinetics of RU-0211 after oral administration to rats and mice	RTU/SR01-068,069,070	--	--	38
Pharmacokinetics of RU-0211 after single oral Administration to mice#	EIH108	--	--	39
Pharmacokinetics of RU-0211 after single oral administration in female rabbit#	EIH118-1	--	--	40
Pharmacokinetics of RU-0211after oral administration to monkey#	EIH106	--	--	41
Pharmacokinetics of RU-0211 after single oral administration to dog#	EIH107	--	--	42
Distribution				
Tissue distribution of radioactivity in rats after single oral administration of ^{3}H-RU-0211	RTU/SR01-080	--	--	43
Plasma protein binding of RU-0211 in the rat, dog and human samples	--	--	--	45

Plasma protein binding in rats after oral administration of ^{3}H-RU-0211#	SPI/SR02-022	--	--	48
Metabolism Metabolites of RU-0211 after oral administration to rat, dog, monkey and mouse#	EIH103-4	--	--	48
In vitro metabolism of RU-0211-Comparative metabolism study using rat, dog and human liver microsomes	RTU/SR01-077	--	--	53
Metabolism in gastrointestinal tissues after single administration of RU-0211 to rats#	EIH119	--	--	54
Metabolite identification of RU-0211 after oral administration in rats#	EIH120	--	--	56
Metabolite profiling in rat and dog plasma after Oral administration of RU-0211#	SPI/SR05-002	--	--	61
Evaluation of Cytochrome P450 involved in the biotransformation of RU-0211 to 15-hydroxy-RU-0211#	SPI/SR05/006	--	--	65
In vitro human Cytochrome P450 inhibitory drug -drug interaction study with SPI-0211#	SPI/SR04-009	--	--	70
In vitro assessment of the enzyme induction potential of RU-0211 in primary cultures of human hepatocytes#	SPI/SR04-018	--	--	70
Investigation of involvement of microsomal carbonyl reductase in the biotransformation of RU-0211 to 15-hydroxy-RU-0211#	SPI/SR05-015	--	--	71
HPLC profiles of metabolite M3 in human plasma and peak C in rat plasma after oral administration of ^{3}H-RU-0211#	EIH103-A1	--	--	73

Acute Toxicity Study: Single dose oral toxicity studies in rats and dogs	403415 600116	# 4 & # 5		74
Subacute/Subchronic/Chronic Toxicity Study:				
Rat:				
2-week oral toxicity study #	500915	# 4		77
4-week oral toxicity study	500116	#5		80
26-week oral toxicity study	550218	#9		86
Mouse:				
13-week oral toxicity study	YK-Ca-1001	#9		91
Dog:				
2-week oral toxicity study #	640219	#5		95
4-week oral toxicity study	640314	#5		97
39-week oral toxicity study	670119	#9		103
Genetic Toxicology Study				
Bacterial reverse mutation (Ames) assay	900915	# 4		109
Chromosomal aberration assay in cultured mammalian (CHL) cells	971415	#4 and # 5		112
Mouse lymphoma cell forward gene mutation assay	RTU/SR01-031	#5		117
Mouse bone marrow micronucleus assay	940315	# 4		120
Carcinogenicity Studies:				
104-week oral carcinogenicity study in mice	7142-109	#020212, #021007, #021203		126
104-week oral carcinogenicity study in rats	7142-100	#020108, #020109,		139

		#0201702		
Reproductive and Developmental Toxicology:				
Segment I (oral) fertility and reproductive performance study in rats	100116	#6		154
Segment II (oral) teratogenicity study in rats	RTU/SR1-062	001121-4		159
Segment II (oral) teratogenicity study in rabbits	SPI/SR03-039	YY020902-904		165
Segment III (oral) pre- and post-natal development study in rats	SPI/SR02-010	010221-010224		171
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Special Toxicology Studies:				
Dose-range toxicity study in guinea pigs#	4619-001	--		178
Abortifacient study in Rhesus monkeys#	SBL99-46	#12		187
Abortifacient study in guinea pigs	4619-002	YY040621-1		181

#, New studies submitted and were reviewed under NDA 21-908.

Studies not reviewed within this submission:

Following analytical methods and validation reports were not reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

RU-0211 is a prostaglandin E1 analog. It is a specific activator of CIC-2 chloride channels, which is involved in the secretion of fluids in the gastrointestinal tract. In human intestinal epithelial (T84) cells, it caused a dose-dependent increase in chloride current. RU-0211 caused a dose-dependent augmentation of acetylcholine-induced contractions of isolated rat ileum. RU-0211 caused a dose-dependent increase in intestinal fluid secretion in rats. It had no effects on the serum levels of Na^+, K^+ and Cl^- in rats at oral doses up to 100 µg/kg. The main metabolite of RU-0211, 15-hydroxy-RU-0211 (M3) also caused a dose-dependent increase in the intestinal fluid secretion in rats, with a potency similar to that of the parent compound. RU-0211 at oral doses of 1 to 100 µg/kg, caused an increase in intestinal transit in morphine-treated mice without affecting the analgesic effects of morphine. It had no effects on the respiration rate, heart rate or ECG parameters of anesthetized dogs at intraduodenal doses up to 1000 µg/kg. It had no effect on the central nervous system of rats at oral doses up to 1000 µg/kg. RU-0211 did not cause a prolongation of action potentials in canine isolated canine cardiac Purkinje fibers.

2.6.2.2 Primary pharmacodynamics

Effect of RU-0211 on EP (Prostaglandin E) Receptors and FP (Prostaglandin F) Receptor – Bioassay Using Smooth Muscle (Study Report # RTU/SR00-032)

The effects of RU-0211 on prostaglandin E receptors (EP_1, EP_2 and EP_3) and the prostaglandin F receptor (FP) were examined on smooth muscle preparations containing the specific receptor. Guinea pig ileum longitudinal and circular muscle preparations were used to examine EP_1 and EP_2 activities, and guinea pig isolated vas deferens was used to assess EP_3 activity. Dog iris sphincter smooth muscle contraction was used to assess FP receptor activity.

Guinea pig ileum longitudinal smooth muscles, suspended in organ bath, contracted dose-dependently in response to RU-0211 (10^{-8} to 10^{-5} M). The contraction caused by RU-0211 was expressed as the percentage of contraction caused by $1x10^{-5}$ M prostaglandin E_2 (PGE_2) and the ED_{50} calculated.

Inhibition of electrical field stimulation induced contraction of guinea pig ileum circular smooth muscle was used to measure EP_2 receptor activity of RU-0211 (10^{-9} to 10^{-5} M) and expressed as the percentage of inhibition caused by $1x10^{-5}$ M PGE_2.

The effect of RU-0211 (10^{-9} to $3x10^{-7}$ M) on EP_3 receptors was investigated by measuring its ability to inhibit the electrical field stimulation induced contraction of the guinea pig vas deferens. The inhibitory activity was expressed as the percentage of that caused by $1x10^{-6}$ M PGE_2.

Contraction of dog iris sphincter smooth muscle in response to RU-0211 (10^{-9} to 10^{-5} M) was measured in an organ bath and was expressed as the percentage of contraction caused by $1x10^{-7}$ M $PGF_{2\alpha}$.

RU-0211 caused contraction of the guinea pig ileum longitudinal muscle. At 10 μM concentration, it caused 54.1% of the maximal contraction caused by PGE_2. Misoprostol, at the same concentration, caused 49.8% contraction. In the guinea pig ileum circular smooth muscle, RU-0211 caused an inhibition of the electrical stimulation induced contraction with an IC_{50} value of 581.4 nM. The IC_{50} values for PGE_1 and PGE_2 were 28.2 and 52.2 nM respectively. The IC_{50} value for inhibition of electrical field stimulation-induced contraction of guinea pig vas deferens by RU-0211 was 40.6 nM, while PGE_1 and PGE_2 had IC_{50} values of 1.4 and 1.9 nM respectively. Misoprostol has similar potency to PGE_1 and PGE_2 (IC_{50} value 0.9 nM). RU-0211 had very low affinity for FP receptors, as it produced very weak contraction of the dog iris sphincter smooth muscle (EC_{50}, 10,000 nM). $PGF_{2\alpha}$, on the other hand, was a potent agonist in this preparation (EC_{50}, 4.5 nM), while PGE_2 was a weaker agonist (EC_{50}, 403 nM). The effects of PGE_1, PGE_2, PGF_{2a}, misoprostol and RU-0211 on (G. pig ileum longitudinal (EP_1) and circular (EP_2) muscles, G. pig vas deferens (EP_3) and dog iris sphincter (FP) muscles are shown in the figures below.

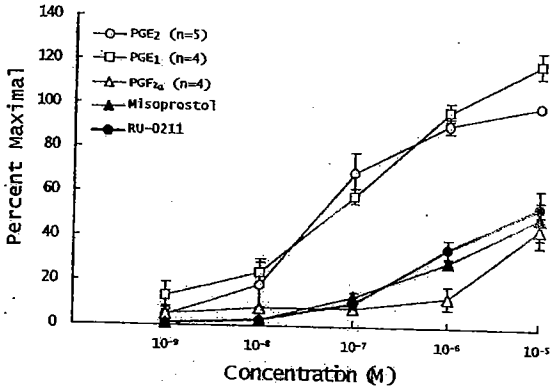


Figure 1. Dose response curve for the stimulation of contractions in the longitudinal muscle layer of isolated segments of guinea pig ileum. Data represent the mean ± S.E. of 3 to 5 determinations.

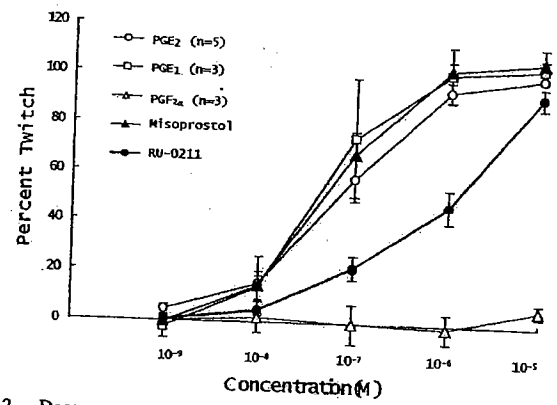


Figure 2. Dose response curve for the inhibition of electrically stimulated twitch contractions in circular muscle layer of isolated segments of guinea pig ileum. Data represent the mean ± S.E. of 3 to 5 determinations.

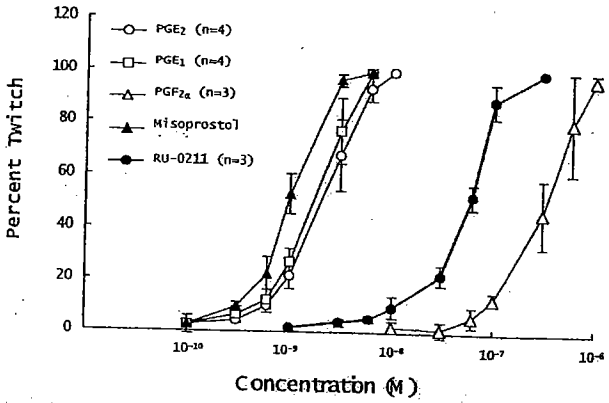


Figure 3. Dose response curve for the inhibition of electrically stimulated twitch contraction in isolated segments of guinea pig vas deferens. Data represent the mean ± S.E. of 3 to 4 determinations.

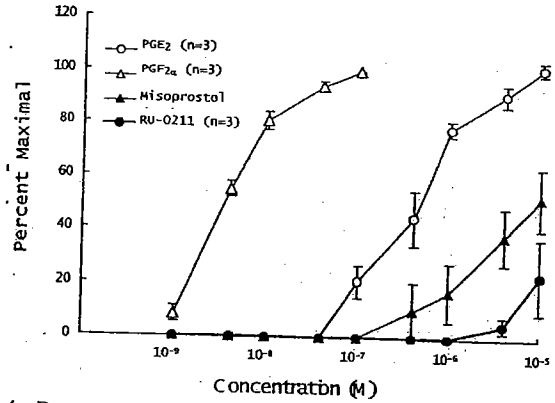


Figure 4. Dose response curve for the stimulation of contractions in the iris sphincter segments of beagle dogs. Data represent the mean ± S.E. of 3 determinations.

Thus, similar to misoprostol, RU-0211 had high affinities for EP_2 and EP_3 receptors and low affinities for EP_1 and FP receptors. RU-0211 and misoprostol had similar affinities for EP_1 receptors, while misoprostol had higher affinities for EP_2 receptors.

Effects of RU-0211 on Recombinant and Native Intestinal Cell CIC-2 Cl^- Channels (Study # SPI/SR02-009)

Methods: The mechanism of Cl^- secretion by RU-0211 was examined by measuring the short circuit current (I_{sc}) in T_{84} human gastrointestinal epithelial cells and by measuring Cl^- transport in human epithelial kidney (HEK) cells by whole cell patch clamp technique. T_{84} cell monolayers were grown in confluence with an air-liquid interface on — inserts coated with collagen. CIC-2 and CFTR transfected HEK cells were grown in culture in NEM medium, supplemented with heat-inactivated horse serum. Measurements of short circuit current in T_{84} cells were performed using a 742C voltage clamp device. Changes in the short circuit current (ΔI_{sc}) after treatment with RU-0211 were normalized to filter area and expressed as $\Delta I_{sc}/cm^2$. In the whole cell patch clamp in HEK cells, currents were elicited by voltage clamp pulses between +40 mV and –140 mV. Currents were measured 50-100 ms after start of the pulse. RU-0211 was diluted in DMSO and added to the bath at concentrations ranging from 1 nM – 10 μM. Current-voltage (I-V) values for Cl^- currents were normalized to cell membrane area (capacitance; pA/pF).

Results:

Short circuit current studies in T_{84} cells: Under the conditions of the experiment, the increases in the short circuit current (ΔI_{sc}) at the steady state in T_{84} cells is proportional to Cl^- current. Treatment of the cells with RU-0211 caused concentration-dependent changes in the short circuit current. The 50% effective concentration (EC_{50}) of RU-0211 was determined to be 13.0 ± 3.30 nM. The effect of RU-0211 on short circuit Cl^- currents in T_{84} cells is summarized in the Table below.

RU-0211 Concentration (nM)	ΔI_{sc} (μA/cm^2 (Mean ± SEM)	$1/\Delta I_{sc}$	1/RU-0211 Concentration
10 (n=3)	26.2 ± 6.6	0.038	0.10
20 (n=3)	39.3 ± 3.6	0.025	0.05
50 (n=3)	56.5 ± 1.3	0.018	0.02
250 (n=3)	59.6 ± 8.8	0.017	0.004

Patch Clamp Studies: Patch clamp studies were conducted in non-transfected and CIC-2 and CFTR transfected HEK cells. Non-transfected cells did not exhibit any Cl^- channel activity and it was not changed after treatment with RU-0211. RU-0211 caused a dose-dependent increase in the Cl^- conductance in CIC-2 transfected cells with an EC_{50} of 25.1 ± 6.7 nM. The linear double reciprocal

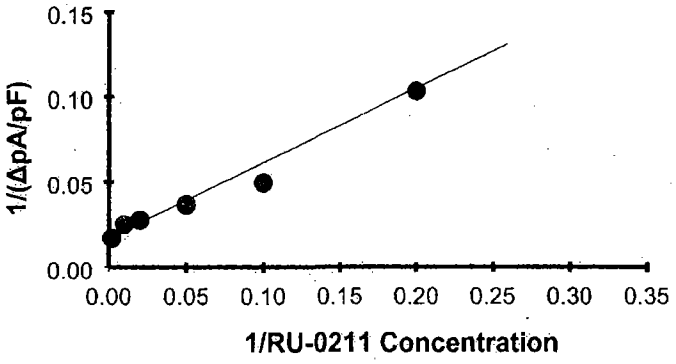
plot of the dose-response data indicated that RU-0211 activation was saturable with a single component of activation. RU-0211 induced CIC-2 Cl^- channel activation was not mediated via a protein kinase A (PKA)-dependent mechanism, because the current was not inhibited by a specific PKA inhibitor. The effects of RU-0211 on Cl^- currents in transfected HEK cells are summarized in the sponsor's Table below.

Table 4.
Summarized Data for Dose Response of RU-0211 on Human CIC-2 Cl^- Currents in Stably Transfected HEK Cells by Patch Clamp.

RU-0211 Concentration (nM)	*N*	*pA/pF*[a]	*ΔpA/pF*	*1/(ΔpA/pF)*	*1/RU-0211 Concentration*
Control	3	8.7 ± 1.2	0.0		
5	3	18.4 ± 3.1	9.7	0.100	0.2
10	3	28.9 ± 1.4	20.2	0.049	0.1
20	3	35.9 ± 2.9	27.2	0.037	0.05
50	3	44.7 ± 2.7	36.0	0.028	0.02
100	3	48.5 ± 2.7	39.8	0.025	0.01
500	3	67.9 ± 4.0	59.2	0.017	0.002

[a] Mean ± SEM

The double reciprocal plot of 1/(ΔpA/pF) against 1/RU-0211 concentration is shown in the sponsor's Figure below.



To rule out the possibility of RU-0211 activation of chloride currents in CFTR chloride channels, the effect of RU-0211 on chloride currents was also examined in CFTR-transfected HEK cells. However, RU-0211 was not capable of stimulating CFTR Cl^- channel activity in these cells.

In summary, the mechanism of chloride secretory effect of RU-0211 was examined by its effect on chloride transport in native and recombinant cells by using both short circuit current and whole cell patch clamp technique. Short circuit current, measured in T_{84} intestinal epithelial cells, which is proportional to Cl^- current, was increased dose-dependently by RU-0211. The data suggest that RU-0211 is an activator of chloride channels in T_{84} intestinal epithelial cells.

To identify the specific types of chloride channels activated by RU-0211, patch clamp studies were conducted in CIC-2 and CFTR chloride channel transfected HEK cells. Treatment with RU-0211 caused a dose-dependent increase in Cl^- current in the CIC-2 transfected cells, but not in the CFTR transfected cells. This suggests that RU-0211 is a specific activator of CIC-2 Cl^- channels and the CIC-2 Cl^- channels may play a role in RU-0211 induced Cl^- transport in the intestinal epithelium. RU-0211-induced chloride channel activation was independent of activation by protein kinase A (PKA), which is different from PGE_1, a PKA-dependent chloride channel activator.

Effect of RU-0211 on isolated rat ileum

The effect of RU-0211 on isolated rat ileum was examined after suspending the ileum in organ baths containing oxygenated Tyrode solution at 30^0C. The contractions of the muscles were recorded with a thermal tip recorder through an isometric recorder. RU-0211 was dissolved in physiological saline containing 5% ethanol and 0.03% polysorbate 80. The concentrations tested were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} g/ml. The contractions were expressed as the percentage of maximum contraction caused by 10^{-6} g/ml of acetylcholine.

RU-0211 caused dose-dependent increase in contractions of the isolated rat ileum strips (at doses of 10^{-7}, 10^{-6} and 10^{-5} g/ml, it caused 19, 47 and 67% increase in contractions respectively). The lower concentrations had no effect on the rat ileum. The other two cyclic fatty acid derivatives, alprostadil (PGE_1) and limaprost (both at $3x10^{-4}$ g/ml) also caused contractions of the ileum. When 10^{-6} g/ml of acetylcholine was added 5 minutes after RU-0211 (10^{-6} or 10^{-5} g/ml), the contractions caused by RU-0211 were significantly higher than vehicle controls suggesting that RU also caused augmentation of acetylcholine induced contractions.

In vivo studies:

Effect of RU-0211 on Intestinal Fluid Secretion

The effects of RU-0211 on intestinal fluid secretion were evaluated in male Wister rats at oral doses of 0.1, 0.5 and 1.0 μg/kg (5 ml/kg in distilled water). Control animals received distilled water only. Thirty minutes after RU-0211 or distilled water administration, the animals were sacrificed, abdomens opened and tied at the first portion of the duodenum and end portion of the ileum. The intestine was then removed and the volume of fluid in each intestine was measured.

RU-0211 caused an increase in the intestinal fluid secretion at 0.5 and 1.0 μg/kg doses in a dose-dependent manner. The total intestinal fluid volume increased from 1.2±0.06 ml (control) to 1.7±0.12 ml and 2.0±0.15 ml at 0.5 and 1.0 μg/kg doses respectively. The lower dose (0.1 μg/kg) had no effect on the intestinal fluid secretion in the rat. The estimated ED_{50} of RU-0211 in stimulating intestinal fluid secretion

in rats was 0.6 μg/kg. As compared to PGE_2, RU-0211 was about 100 times more potent in causing an increase in the intestinal fluid volume in rats.

Water Excreting Effect of RU-0211 into the Bowel of Rats

The effect of oral RU-0211 on the excretion of water into the bowel of rats was examined in fasted Wister rats. The animals received IV injections of tritium water (9,300,000 dpm/ml in saline). One minute after the injection of tritium water, RU-0211 (10 μg/kg; 5 ml/kg) was administered orally. The control animals received the same volume of the vehicle (distilled water containing 0.01% Tween 80). Thirty minutes after RU-0211 or the vehicle administration, the animals were sacrificed, abdomen opened and tied at the first portion of the duodenum and the end portion of the ileum. The intestinal fluid was collected and weighed. The radioactivity of the fluid collected was measured using a scintillation counter.

Oral administration of RU-0211 to rats caused an increase in both the intestinal fluid weight (161.8%) and the level of radioactivity (197.7%) ($p<0.01$) as compared with those receiving the vehicle. The weight of the intestinal fluid in the controls was 0.89±0.16 g and those receiving RU-0211 was 2.33±0.18 g. The radioactivity in the intestinal fluid increased from 50,894±9,225 dpm (in control) to 151,530±1,221 dpm (in treatment group). Thus, RU-0211 caused significant augmentation of the secretion of fluid into the rat intestine.

Effects of RU-0211 on Secretion of Electrolytes into the Bowel of Rats (mechanism of stimulation of intestinal fluid secretion):

To investigate the mechanism of intestinal fluid secretion by RU-0211 in rats, the effects of the compound on secretion of electrolytes into the bowel were studied. The animals received RU-0211 at 1, 10 and 100 μg/kg doses. Thirty minutes after dosing, blood samples were collected from the abdominal aorta and the animals sacrificed to collect the intestinal fluid. The volume of the intestinal fluid was measured and the Na^+, K^+ and Cl^- concentrations in both the blood and the intestinal fluid were measured.

Oral administration of RU-0211 caused significant dose-dependent increases in the intestinal fluid in rats at 1,10 and 100 μg/kg doses (1.7, 3.7 and 5.9 times increases respectively). The concentrations of Na^+ and K^+ in the intestinal fluids were significantly decreased while the concentrations of Cl^- were increased in a dose-dependent manner by RU-0211. Based on the total intestinal fluid volume and the concentrations of the electrolytes, the total amounts of Na^+, K^+ and Cl^- in the intestinal fluid were significantly higher in RU-0211 treated animals as compared with the controls. In contrast to its effects on the intestinal fluid electrolytes, RU-0211 had no effect on the serum levels of Na^+, K^+ and Cl^- at oral doses up to 100 μg/kg. Thus, the Cl^- secretion, stimulated by RU-0211 may play an important role in

causing fluid secretion into the intestine. The data for the effect of RU-0211 on the intestinal fluid volume and electrolytes concentration is shown in the sponsor-provided table below.

Effect of RU-0211 on the volume of the intestinal fluid and Na^+, K^+ and Cl^- content in the intestinal fluid of rats

Group		n	Volume	Na^+		K^+		Cl^-	
				concentration	total amount	concentration	total amount	concentration	total amount
			mL	mEq/L	mEq	mEq/L	mEq	mEq/L	mEq
Control	(vehicle)	7	0.9±0.1	210±13	0.18±0.02	21.1±2.4	0.018±0.002	41.8±3.9	0.037±0.006
RU-0211	1μg/kg p.o.	7	1.5±0.2	185±4	0.27±0.03	19.7±1.2	0.028±0.004	82.2±7.0**	0.127±0.030
RU-0211	10μg/kg p.o.	7	3.3±0.3**	158±2##	0.52±0.05**	13.6±0.6#	0.044±0.003**	110.1±5.6**	0.372±0.044**
RU-0211	100μg/kg p.o.	7	5.3±0.2**	157±1##	0.83±0.04**	12.5±0.5##	0.066±0.004**	126.6±2.4**	0.670±0.023**

vehicle : distilled water containing 0.5% ethanol and 0.01% polysorbate 80, Mean±S.E.,

** $P<0.01$ (increase) , # $p<0.05$, ## $p<0.01$ (decrease) compared with control group (Dunnett's test)

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Effects of RU-0211 on Morphine-Induced Constipation and Analgesia:

The effects of RU-0211 on morphine-induced constipation and analgesia were evaluated in ICR mice. The mice received intraperitonial injections of morphine (5 mg/kg). Immediately after the injections, the animals received orally a graphite marker (0.1 ml). The animals were treated orally with either the vehicle (physiological saline containing 0.5% ethanol and 0.01% polysorbate 80) or 1, 10 or 100 µg/kg RU-0211 (5 ml/kg). A normal control group received the vehicle and the graphite marker without morphine. All animals were sacrificed 150 min after administration of the graphite markers and the presence of the marker in the cecum was evaluated. The effects of RU-0211 (0.1, 1, 10 and 100 µg/kg) on the analgesic effects of i.p. morphine (5 mg/kg) were evaluated in mice by measuring the responses following tail-pinch.

Graphite markers were present in the cecum of all mice in the normal control group. In the morphine-treated group, graphite marker was present only in 20% (3 of 15) of the animals. In animals that received 0.1, 1, 10 and 100 µg/kg doses of oral RU-0211 after morphine, had graphite markers in the cecum of 27% (4/15), 60% (9/15), 87% (13/15) and 93% (14/15) of the animals respectively. Thus, RU-0211, at oral doses of 1, 10 and 100 µg/kg caused significant increases in the intestinal transit of the graphite markers in the morphine-treated mice as compared with the morphine-treated controls. Morphine caused significant analgesia in the animals as determined by an increase in the response time to tail-pinch. The analgesic effect of morphine was not affected by oral RU-0211 at doses up to 100 µg/kg.

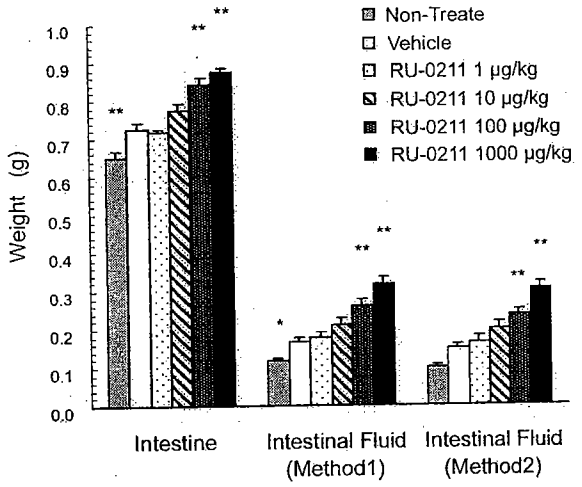
The effects of three commonly used laxatives (sennoside A.B, sodium picosulfate and magnesium oxide) on morphine-induced slowing of transit of the graphite markers were also examined in this animal model. Sennoside A.B had no effect on the morphine-induced constipation in mice at doses up to 48 mg/kg. Sodium picosulfite (15 mg/kg) and magnesium oxide (400 mg/kg) were effective in reducing morphine-induced constipation in mice.

Enteropooling Effects of RU-0211 in C57BL/6 Mice – Oral Administration (Study Report # SPI/SR02-017)

Fasted male C57BL/6 (C57BL/6N – SPF) mice were used in the study. Each animal received a single dose of RU-0211 or the vehicle (1% polysorbate 80 solution), administered by oral gavage (20 ml/kg). For the time course study, 10 mice were administered 100 µg/kg of RU-0211 and 5 animals each were sacrificed at 10, 20, 30 and 45 minutes following administration. In the dose-response study, 5 mice each were administered 1, 10, 100 and 1000 µg/kg of RU-0211, and the animals were sacrificed 10 minutes post-dose. Intestinal fluid weight was determined by two different methods. Following administration of RU-0211 or the vehicle, the small intestine was excised and weighed (W1). The small intestine was then placed on a pre-weighed paper towel and the intestinal fluid was expelled using a roller. In method 1, the small intestine was re-weighed (W2) and the resulting weight was subtracted from the initial weight of the intestine

(W1). In method 2, the combined weight of the paper towel and the roller before use (W3) was subtracted from the paper towel and the roller following expulsion of intestinal fluid.

In the time course study, the administration of a 100 µg/kg dose of RU-0211 to C57BL/6 mice significantly increased the weight of the small intestine at 10 min post-dose, when compared with the vehicle. No significant increase in the intestinal weight was observed at 20, 30 and 40 min post-treatment. Based on the results of the time-course study, a dose-response study was conducted using 10 min post-dosing as the end point. Compared to the vehicle, RU-0211 caused a dose-dependent increase in the weight of the small intestine which was due to increased intestinal fluid weight. At 100 and 1000 µg/kg doses, 1.6-fold and 2.0-fold increases in intestinal weights over the control weights were observed. The findings were similar in both methods of measurement. The effects of different doses of RU-0211 on the weight of intestine and intestinal fluid weights are shown in the Figure below.

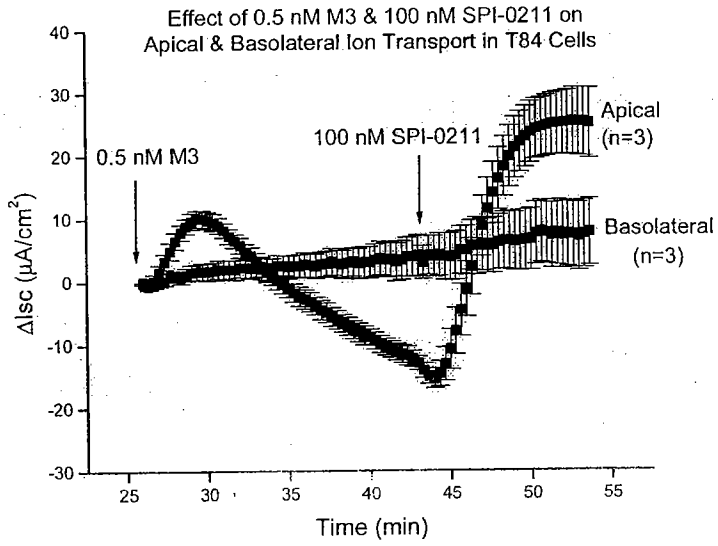


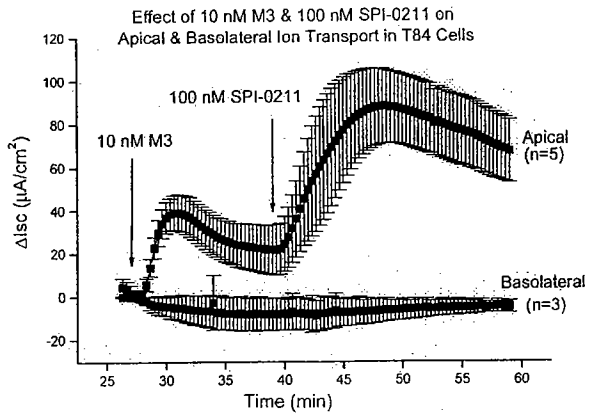
Other Studies:

Effect of M3, a SPI-0211 Metabolite, on Apical and Basolateral Ion Currents in T84 Cells (Study Report # SPI/SR05-018)

It has been shown that SRI-0211 (RU-0211) activates T84 cell chloride transport and CIC-2 chloride channels in HEK-293 cells. It is thought that local activation of the CIC-2 chloride channels, which are present on the apical side of the intestinal membrane, leads to the generation of chloride-rich intestinal fluid. The study was designed to determine of M3, a metabolite of SPI-0211 has any effect on the basolateral membrane ion currents in T84 cells (a human intestinal cell line). The cells were grown to confluence on filter supports, and the effects of M3 (0.5 and 10 nM) and SPI-0211 (100 nM) on the short circuit current (I_{sc}) was measured. Apical and basolateral membrane currents were measured after permeabilization of the membranes with nystatin (200 µg/ml).

The EC50 value for the whole cell patch clamp studies of recombinant CIC-2 in HEK cells was similar to the EC50 for activation of chloride currents by SPI-0211 in nystatin-permeabilized T84 cells (approximately 40 nM). When the apical membrane was permeabilized with nystatin, SPI-0211 or M3 had no effect on T84 cell basolateral membrane ion currents (I_{sc}). When the basolateral membrane was permeabilized with nystatin, 0.5 nM M3 caused a rapid transient increase in I_{sc} (approximately 10 μA followed by a rapid decrease to below baseline within 10 minutes. This could be due to further metabolism of M3. Subsequent addition of 100 nM SPI-0211 caused an additional increase in I_{sc} by about 40 μA across apical membrane. The response to SPI-0211 was sustained for a longer period of time than the response to M3. At the 10 nM concentration, M3 caused a larger increase in I_{sc} as compared with that at 10 nM, and the response was sustained for a longer period. Addition of 100 nM SPI-0211 after 10 nM M3 caused an additional increase in I_{sc} across apical membrane by about 50 μA. The overall increase by 0.5 nM or 10 nM M3 plus 100 nM SPI-0211 was similar, suggesting that M3 did not cause a down regulation of chloride ion channels. The effects of M3 (0.5 nM and 10 nM) on apical and basolateral ion transport in T84 cells is shown in the Figures below.





Thus, the results of the study suggest that both M3 and SPI-0211 activate chloride channels that are found on the luminal (apical) side of the intestine.

Enteropooling Effects of the RU-0211 Metabolite M3 (U-E230) – Oral Administration (Study Report #SPI/SR03-033; Cross reference #YK-En-2014).

After oral administration of RU-0211 to healthy human volunteers, M3 (U-E230) was identified as one of the major metabolites. M3 is formed by the reduction of the C-15 carbonyl group of RU-0211. M3 was also detected in mice, dogs and rats. In the present study, the enteropooling effects (intestinal fluid accumulation) of M3 were examined in male Wistar rats after oral administration of 0, 0.03, 0.1, 0.3, 1 or 3 μg/kg doses (5 ml/kg). The effects were compared with those of same doses of RU-0211. At 30 minutes after administration of the agents, the small intestine (from the beginning of the duodenum to the end of ileum) was removed after ligation, and the fluid accumulated in the intestinal segment was quantified.

The intestinal fluid level of the control animals was 0.83±0.09 ml, and after 0.03, 0.1, 0.3, 1 and 3 μg/kg doses, the fluid levels were 0.65±0.05, 0.92±0.11, 0.97±0.10, 1.22±0.14 and 2.26±0.24 ml, respectively (significant only at 3 μg/kg). RU-0211 also caused similar increase in the intestinal fluid accumulation that was significant at 3 μg/kg. The enteropooling effects of U-E230 and RU-0211 in rats after oral administration are shown in the Table below.

Enteropooling Effects of U-E230 and RU-0211 after Oral Administration in Rats

Group	Dose (μg/kg)	No. of Animals	Intestinal Fluid Volume[a] (mL)	% Increase[b]	ED_{50} Value (μg/kg)
Vehicle Control	---	6	0.83 ± 0.09	---	---
U-E230	0.03	5	0.65 ± 0.05	-22.0	0.49
	0.1	5	0.92 ± 0.11	+10.4	
	0.3	5	0.97 0	+16.4	
	1	5	1.22 ± 0.14	+46.4	
	3	4	2.26 ± 0.24[c]	+172	
RU-0211	0.03	5	0.87 ± 0.11	+4.40	0.64
	0.1	5	0.96 ± 0.20	+15.2	
	0.3	5	1.06 ± 0.19	+27.2	
	1	5	1.35 ± 0.19	+62.0	
	3	6	2.51 ± 0.25[c]	+201	

[a] Mean ± SE

[b] Calculated as:

$$\% \text{ increase} = \left(\frac{\text{mean of intestinal fluid volumes in test substance treated group}}{\text{mean of intestinal fluid volumes in vehicle control group}} - 1 \right) \times 100$$

[c] $p<0.01$ vs vehicle control (Dunnett's test)

Thus, similar to RU-0211, its metabolite M3 (15-hydroxy-RU-0211) caused intestinal fluid accumulation in rats after oral dosing.

Enteropooling Effects of 15a-OH and 15ß-OH Epimers of RU-0211 Metabolite M3 (U-E230) – Oral Administration (Study #YK-En-2016)

In humans, 15-hydroxy-RU-0211 (M3 or U-E230) was identified as the main metabolite in the plasma. As U-E230 exists as a mixture of 15a-OH (U-E231) and 15ß-OH (U-E232) epimers, the enteropooling effects of the two epimers were examined in male Wistar rats following oral administration. Rats were administered (gavage) the vehicle or the epimers at 0.03, 0.1, 0.3, 1, 3 or 10 µg/kg doses. Intestinal fluid volume was measured in a ligated segment of the intestine 30 minutes after dosing. As a positive control, the enteropooling effects of RU-0211 (3 µg/kg) was also examined.

The 15a-OH epimer (U-E231) had no effects on the intestinal fluid levels in rats following oral administration at doses up to 10 µg/kg. On the other hand, the 15ß-OH epimer (U-E232) caused a dose-dependent increase in the intestinal fluid levels that was significant at 1, 3 and 10 µg/kg doses. At 1, 3 and 10 µg/kg doses, U-E232 increased intestinal fluid levels by 96.8%, 165% and 178% over the vehicle control. Based on the dose-response curve of U-E232, the dose producing 50% increase in intestinal fluid (ED_{50}) was calculated as 0.33 µg/kg. RU-0211, administered at a dose of 3 µg/kg, produced 118% increase in the intestinal fluid levels. The enteropooling effects of U-E231, U-E232 and RU-0211 in male rats following oral administration are shown in the Table below.

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Enteropooling Effects of U-E231, U-E232 and RU-0211 after Oral Administration in Rats

Group	Dose (µg/kg)	No. of animals	Intestinal Fluid Volume[a] (mL)	% Increase[b]	Dose producing a 50% increasase (µg/kg)
Vehicle	---	6	1.04 ± 0.20	---	---
U-E231	0.03	6	0.91 ± 0.14	-12.8	>10
	0.1	5	0.70 ± 0.11	- 32.8	
	0.3	5	0.82 ± 0.21	- 21.3	
	1	6	0.85 ± 0.11	- 18.4	
	3	6	1.18 ± 0.41	13.6	
	10	6	0.68 ± 0.10	- 34.4	
U-E232	0.03	6	0.84 ± 0.17	- 19.2	0.33
	0.1	5	1.08 ± 0.16	4.0	
	0.3	6	1.40 ± 0.32	34.4	
	1	6	2.05 ± 0.32*	96.8	
	3	6	2.76 ± 0.18**	165	
	10	5	2.90 ± 0.28**	178	
RU-0211	3	6	2.28 ± 0.18**	118	---

[a] Mean ± SE.

[b] Calculated as :

$$\% \text{ increase} = \left(\frac{\text{mean of intestinal fluid volume in test substance treated group}}{\text{mean of intestinal fluid volume in control group}} - 1\right) \times 100$$

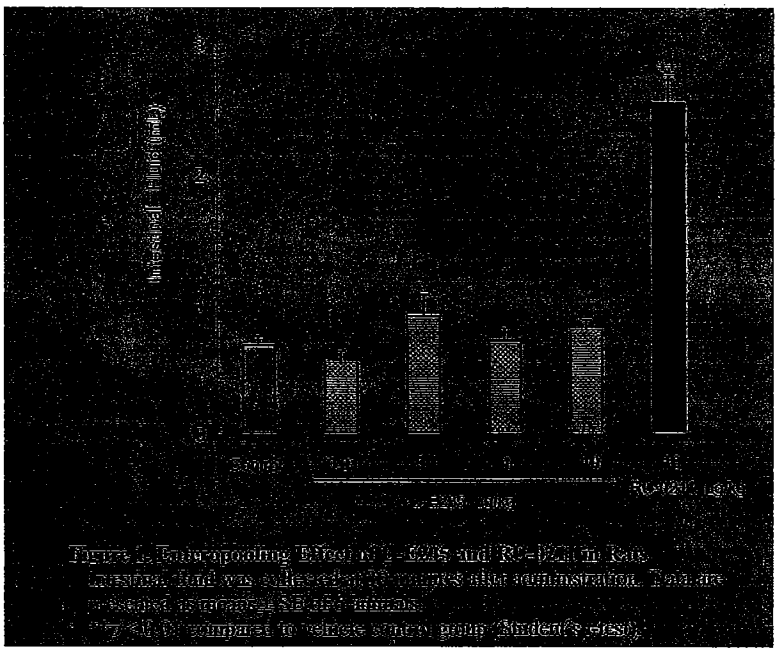
* $p < 0.05$, ** $p < 0.01$ *vs* vehicle control (Dunnett's test)

Thus, the15a-OH epimer of the RU-0211 metabolite (U-E231) was devoid of enteropooling activity in rats, while the 15ß-OH epimer (U-E232) possessed enteropooling activity.

Evaluation of Enteropooling Effect of U-E235, 19-Carboxy-15-Hydroxy-2, 3, 4, 5, 20-Pentanor-RU-0211 in Rats (Study Report #SPI/SR04-016)

The enteropooling effect of U-E235, a pentanor metabolite of RU-0211, was evaluated in male Wistar rats following single oral administration of 0.01, 0.1, 1 and 10 µg/kg doses (6 animals/group). Test agents or the vehicle was administered by oral gavage, and the intestinal fluid accumulation was measured 30 minutes following administration.

A single oral administration of U-E235 at doses of 0.01, 0.1, 1 and 10 µg/kg produced no increase in intestinal fluid volume relative to the vehicle control. RU-0211, on the other hand, produced a significant increase in the intestinal fluid volume compared to the vehicle control. The enteropooling effects of U-E235 and RU-0211 in rats are shown in the Figure below.



Thus, the pentanor metabolite of RU-0211, U-E235 had no enteropooling effect in rats.

Effect of U-E236, a Metabolite of RU-0211, Administered Orally on Intestinal Fluid Secretion in Rats (Study Report #SPI/SR04-017)

The enteropooling effect of U-E236, a metabolite of RU-0211, was evaluated in rats following single oral administration of 0.01, 0.1, 1 and 10 µg/kg doses. The effects were compared with that of a 10 µg/kg dose of RU-0211.

U-E236, at single oral doses of 0.01, 0.1, 1 and 10 µg/kg, produced no increase in intestinal fluid volume in rats. Conversely, a single oral dose of 10 µg/kg RU-0211 produced a significant increase in the intestinal fluid volume in rats.
Thus, the RU-0211 metabolite, U-E236 had no enteropooling effect in rats at oral doses up to 10 µg/kg.

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2.6.2.3 Secondary pharmacodynamics

Effect on isolated guinea pig trachea

The effect of RU-0211 on the guinea pig tracheal smooth muscles was examined after suspending the tracheal muscle strips in Tyrode solution. The test compound was dissolved in physiological saline containing ethanol (5%) and polysorbate 80 (0.03 %) and the final concentrations added in the muscle bath were 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} g/ml. The contractions of the tracheal muscles were expressed as the percent of contraction caused by 10^{-6} g/ml of histamine.

RU-0211 had no significant effect on the isolated guinea pig tracheal smooth muscles at concentrations up to 10^{-5} g/ml.

Effect of RU-0211 on isolated uterus of non-pregnant rats

The effect of RU-0211 was examined on spontaneous movement of the isolated uterus from non-pregnant rats (treated with 20 µg/animal of i.p. estradiol for 2 days), suspended in Lock-Ringer solution in a muscle bath. The concentrations of RU-0211 used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} g/ml.

RU-0211 at 10^{-9} and 10^{-8} g/ml had no effect on the spontaneous contractions of the rat uterine muscles. At 10^{-7}, 10^{-6} and 10^{-5} g/ml, RU-0211 caused 16.8%, 18.4% and 46.3% increase in the amplitudes of the spontaneous contractions of the uterus respectively. The frequency of spontaneous contractions of the rat uterus was increased by 157±23.5%, 189.3±34.1% and 238.0±43.4% respectively at these three concentrations. PGE_1, at concentrations from 10^{-7} to 10^{-5} g/ml also caused similar increases in the amplitude and frequency of contractions of the isolated rat uterus.

Effect of RU-0211 on adenosine 5'-diphosphate (ADP)-induced platelet aggregation

The effect of RU-0211 on the rabbit platelet aggregation induced by ADP was studied *in vitro*. Blood was collected from the rabbits in sodium citrate solution and platelet-rich plasma (PRP) was prepared by centrifugation of the blood. The platelet number in PRP was adjusted to $30x10^4$ cells/µl and 200 µl of PRP was preincubated with the vehicle or the test substance (10^{-7} to10^{-5} g/ml; 25 µl) for 1 min at 37^0C. After 1 min preincubation, the platelet aggregating agent (25 µM ADP) was added. The platelet aggregation was measured with a platelet aggregator meter — . Percent inhibition of aggregation was calculated by comparing with the maximal aggregation in the saline-treatment group.

RU-0211 had no effect on ADP-induced rabbit platelet aggregation at concentrations up to 10^{-5} g/ml. PGE_1 (10^{-7} g/ml), on the other hand, caused significant (72.9%) inhibition of the ADP-induced platelet aggregation.

2.6.2.3 Safety pharmacology

Effect of RU-0211 on the central nervous system

The effects of RU-0211 on spontaneous motor activity, hexobarbital-induced sleeping time and body temperature were examined in male rats. The motor activity was recorded 30 minutes after oral administration of RU-0211. To examine the effect of RU-0211 on hexobarbital-induced sleeping time, i.p. hexobarbital (120 mg/kg) was administered 30 minutes after oral administration of the test substance and the sleeping time was measured.

RU-0211, at oral doses of 10, 100 and 1000 μg/kg, had no effect on the spontaneous motor activity, barbiturate sleep-time or body temperature of rats.

Effect of RU-0211 on respiration, blood pressure, heart rate, blood flow to femoral artery, and ECG in anesthetized dogs

Groups of 3 male beagle dogs (7.7-12.9 kg) were used for the study. Under pentobarbital anesthesia, cannulae were introduced into the trachea, femoral artery, cephalic vein and duodenum. The blood pressure and heart rate were measured by connecting the cannula in the femoral artery to a pressure transducer — . The blood flow in the femoral artery was measured with a blood flowmeter. The respiration rate was measured with a — pneumotachometer and the ECG (standard lead II) was recorded and analyzed with 6-channel animal electrocardiograph. The test material (RU-0211;10,100 and 1000 μg/kg) was administered via the cannula inserted into the duodenum.

RU-0211 had no effect on the respiration rate of dogs at intra-duodenal doses up to 1000 μg/kg. It caused significant fall of the mean arterial blood pressures at 100 (from 131±9 mm Hg to 120±13 mm Hg, 20 min. after dosing; $p<0.05$) and 1000 (from 131±9 mm Hg to 100±3 mm Hg, 20 min. after dosing; $p<0.05$) μg/kg doses. At 1000 μg/kg, the fall in the mean arterial pressure was sustained and lasted for more than 120 minutes. The 10 μg/kg dose had no effect on the mean blood pressure in dogs. The compound had no effect on the heart rate of the dogs at intra-duodenal doses up to 1000 μg/kg. RU-0211 had no significant effect on the blood flow of femoral artery at 10 and 1000 μg/kg doses. However, at 100 μg/kg doses, there was a significant increase in the femoral artery blood flow between 10 min and 45 min

after administration of the drug. RU-0211 had no effect on the ECG parameters of the dogs at intra-duodenal doses up to 1000 μg/kg.

Effects of RU-0211 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (Study # 040713.TVD)

The in vitro effects of RU-0211 on cardiac action potentials were examined in isolated canine Purkinje fibers. Purkinje fibers were isolated from adult canine ventricles using standard methods. RU-0211 was used at 7.5, 75 and 750 pg/ml concentrations, and were added sequentially to four Purkinje fiber preparations at three stimulus intervals (2, 1 and 0.5 ms). Sotalol (100 μM) was used as a positive control. The effects of the drug on action potential parameters were compared with time-matched vehicle control sequences. The following parameters were assessed: resting membrane potential (RMP, mV), action potential amplitude (APA, mV), maximum rate of rise V/s (V_{max}) and action potential duration at 60 and 90% repolarization (APD_{60} and APD_{90}, ms).

RU-0211 caused changes in action potential duration (APD) that ranged from a -2.0% shortening of the ADP_{60} after exposure to 7.5 pg/ml to a 4.2% prolongation of the APD_{60} after exposure to 750 pg/ml. However, RU-0211 did not induce a significant prolongation of APD_{90} or APD_{60} at any of the three stimulus intervals at concentrations up to 750 pg/ml. RU-0211 induced changes in resting membrane potential (RMP) ranged from -1.7 mV hyperpolarization at 75 pg/ml to 1.5 mV depolarization at 7.5 pg/ml. RMP changes were not significant except a 1.1 mV depolarization after exposure to 750 pg/ml. RU-0211 caused reductions in action potential amplitude that ranged from -0.4 mV to -6.0 mV (not significant). At 1 and 0.5 sec basic cycle lengths, that simulate normocardia and tachycardia, respectively, the average change in Vmax were -10.7±7.3%, -6.1±3.5% and -10.9±4.6%, and -6.6±6.1%, -8.7±4.6% and -11.1±6.6% at 7.5, 75 and 750 pg/ml, respectively. The positive control, sotalol caused significant prolongation of the APD_{60} and APD_{90} at all stimulus intervals. The data is summarized in the Tables below.

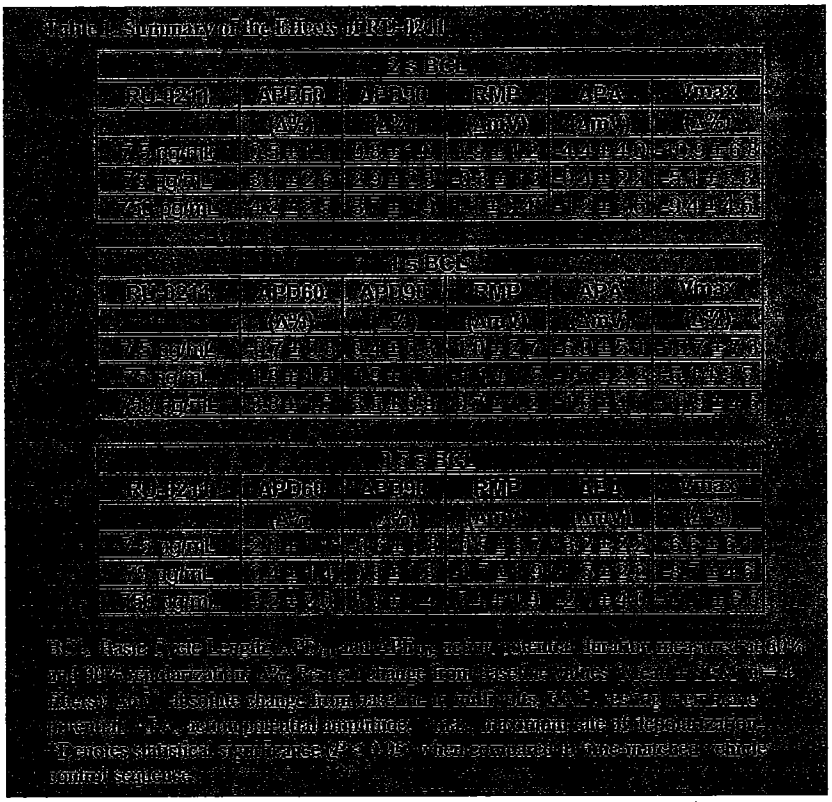

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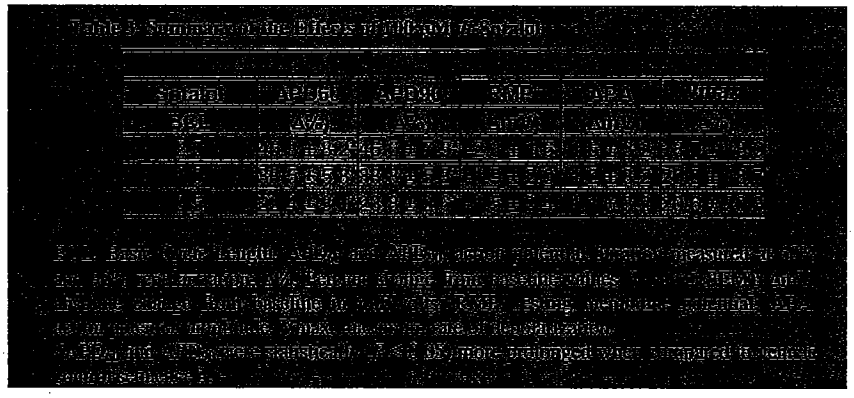
Table 2. Summary of the Effects of Vehicle Control

2 s BCL					
Vehicle Sequence	APD60 (Δ%)	APD90 (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
V1	1.2 ± 1.2	0.9 ± 1.0	-0.1 ± 0.8	-1.7 ± 1.0	-2.6 ± 7.9
V2	2.2 ± 1.8	1.7 ± 1.6	-1.0± 0.4	-1.1 ± 1.0	-2.2 ± 8.7
V3	4.4 ± 2.3	3.0 ± 1.1	-3.4 ± 1.0	1.9 ± 2.7	12.6 ± 19.7

1 s BCL					
Vehicle Sequence	APD60 (Δ%)	APD90 (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
V1	0.5 ± 0.7	0.3 ± 0.7	0.3 ± 0.6	-0.7 ± 1.5	1.4 ± 9.4
V2	3.1 ± 1.7	2.2 ± 1.1	-1.4 ± 0.7	-1.0 ± 0.7	-0.6 ± 10.5
V3	3.1 ± 1.8	2.1 ± 0.6	-2.9 ± 1.2	2.6 ± 1.9	19.4 ± 18.5

0.5 s BCL					
Vehicle Sequence	APD60 (Δ%)	APD90 (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
V1	1.3 ± 0.3	0.9 ± 0.4	0.8 ± 1.0	-0.6 ± 1.4	4.1 ± 10.6
V2	3.0 ± 1.2	1.9 ± 1.0	-2.2 ± 1.5	-1.3 ± 1.8	1.5 ± 12.4
V3	3.3 ± 2.3	2.1 ± 1.1	-2.3 ± 1.5	2.9 ± 1.6	20.3 ± 18.8

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



Thus, RU-0211 did not cause a significant prolongation of action potentials in isolated canine Purkinje fibers.

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Effect on urine volume and urinary excretion of electrolytes in rats

Groups of 10 male rats were used for the study. The test material was administered orally in physiological saline. The volume and Na^+, K^+ and Cl^- concentrations in the urine, collected for 6-hour period, were measured.

RU-0211, at an oral dose of 10 µg/kg, had no effect on the urine volume or urinary excretion of electrolytes. However, at a dose of 100 µg/kg, there was a decrease in urinary excretion of Na^+ (2.13±0.19 meq/kg/6 hr vs. 1.26 meq/kg/6 hr). At 1000 µg/kg, there were decreases in the urinary volume (12.59±2.31ml/kg/6 hr vs. 4.94±0.66 ml/kg/6 hr; $p<0.05$) and urinary excretion of Na^+ (2.13±0.19 meq/kg/6 hr vs. 0.89±0.16 meq/kg/6 hr; $p<0.01$), K^+ (2.37±0.24 meq/kg/6 hr vs. 1.69±0.15 meq/kg/6 hr; $p<0.05$) and Cl^- 2.81±0.22 meq/kg/6 hr vs. 1.61±0.21 meq/kg/6 hr; $p<0.01$).

2.6.2.5 Pharmacodynamic drug interactions

RU-0211 accelerated intestinal transit in animals. Thus, it may interfere with the absorption of certain drugs from intestinal tract by accelerating their transit through the intestine.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

RU-0211 was rapidly absorbed in rats and dogs following oral administration. Following oral administration of single doses of 25, 50 and 100 µg/kg doses to rats, the maximum plasma concentrations were reached in 0.5 to 0.75 hr, and the terminal elimination half-lives ($t_{1/2}$) ranged from 2 to 6 hours. In mice, following oral administration of 25 and 50 µg/kg doses, the T_{max} was 10 to 15 minutes, and the $t_{1/2}$ was about 3 hours. In contrast to rats, in dogs the T_{max} for the total radioactivity was 3 hours following oral administration, and the $t_{1/2}$ was longer than in rats. In rats, there were no signs of accumulation of the drug in any specific tissue. The plasma protein binding of RU-0211 in rats (92 to 95%), dogs (96%) and humans (94-95%) was high. In rats, dogs and mouse, RU-0211 was metabolized rapidly, and several metabolic peaks were detected in the plasma samples. Among them, 15-hydroxy-RU-0211 was the least polar metabolite, and was found be biologically active, with a potency similar to the parent compound. No other metabolite showed the pharmacological activity. In *in vitro* studies with cells expressing different CYP

enzymes, microsomal CYP enzymes had no major role in the metabolism of RU-0211. The parent compound was detected in plasma samples for up to 2 hours after administration. In mice, dogs, rabbits and monkeys, renal excretion was the predominant pathway for elimination of the drug following oral administration. In rats, following oral administration of a radiolabeled dose, major part of the radioactivity was excreted via the fecal route, while following i.v. administration, urinary excretion was higher than fecal excretion.

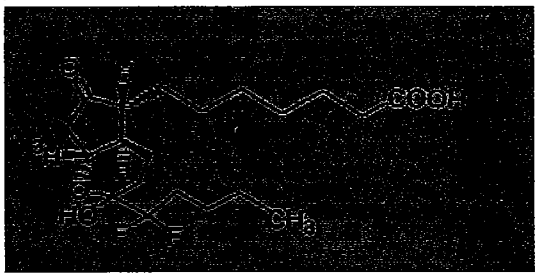
2.6.4.2 Methods of Analysis

RU-0211 or its metabolites in the plasma, urine or feces were analyzed by HPLC,

2.6.4.3 Absorption

Pharmacokinetics of RU-0211 in Rats:

The pharmacokinetic studies with RU-0211 were conducted in male Sprague-Dawley rats after single IV or oral administration of the tritiated compound (specific radioactivity 6.99 mCi/mg; radiochemical purity — as determined by HPLC —). The position of the radiolabel (^3H) in the RU-0211 molecule is on carbon 11 as shown in its structure below.

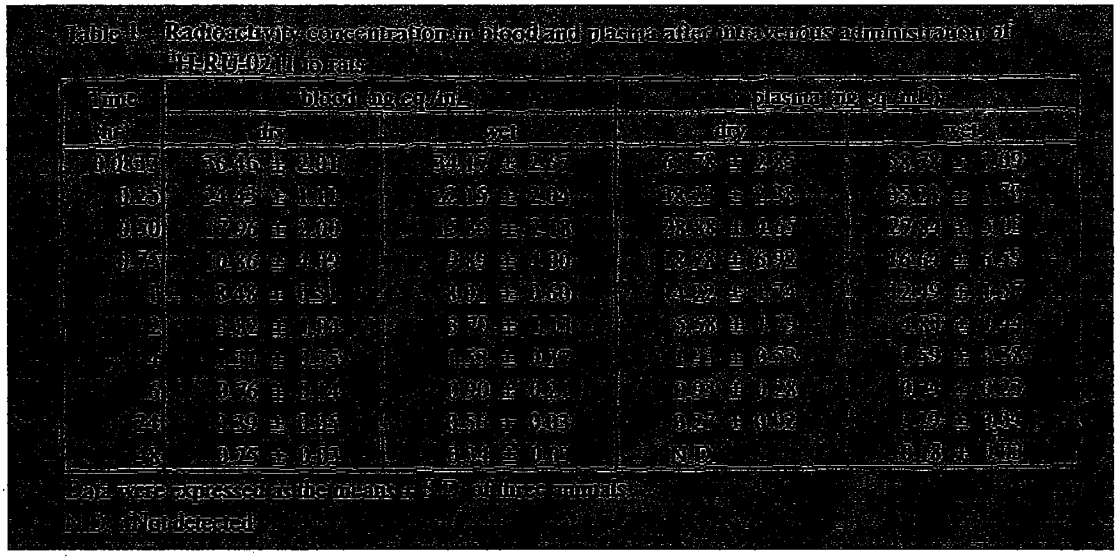


^3H-RU-0211 (Lot # 1) was dissolved in normal saline containing 0.1% polysorbate 80 and was injected into the central vein of right hind leg at 50 µg/kg (2 ml). For oral administration, the labeled compound (appropriately diluted with unlabeled compound and dissolved in medium chain fatty acid triglyceride) was filled into mini-capsules. The mini-capsules were administered orally with the help of a cannula at 25, 50 and 100 µg/kg doses. Blood samples were collected at 5, 15, 30, 45 min, 1, 2, 4, 6, 24 and 48 hr after administration of the radiolabeled compound. The 5-min time-point was not included in the oral dosing studies. Urine and feces excreted at 0-24 hr and 24-48 hr periods after administration of the compound were collected. Cage washings were collected by washing the cage, buttock and tail of the rats 48 hours after administration of the radioactivity. Blood samples were pretreated by wet or dry method. In the dry method, samples of blood/plasma were dried for 12 h at 40^{0}C and solubilized in water and Soluene-350 — isopropanol mixture. In the wet method, the blood sample was solubilized using Soluene 350/isopropanol mixture, decolorized with hydrogen peroxide and heated at 40^{0}C. Urine samples were diluted with water and the feces were homogenized in water. The radioactivity of the samples was counted in a scintillation counter — after adding Scintillator. The $T_{1/2}$ was calculated from

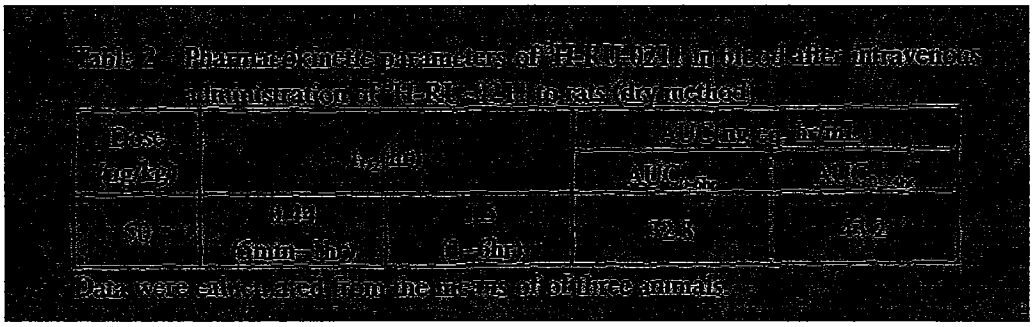
the mean of 3 animals by the least- square linear regression and the AUC values were obtained by the trapezoidal method.

Single IV Dosing:

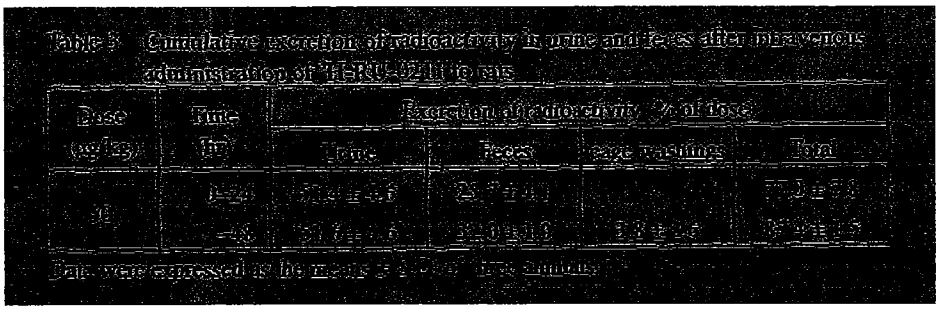
The amount of radioactivity in the blood and plasma after a single IV injection of 50 µg/kg of RU-0211 in rats (using the dry and wet methods) is shown in the sponsor-provided table below.



There were no differences in the blood or plasma radioactivity in rats whether measured by the dry or wet method. The half-lives, determined by the dry method were 0.44 hr (5 min to 1 hr) and 1.5 hr (1-6 hr). The AUC values at 0-6 hr and 0-24 hr were 32.8 ng eq.hr/ml and 43.2 ng eq.hr/ml respectively. The $t_{1/2}$ and AUC values after IV administration of a 50 µg/kg dose in male rats is shown in the sponsor-provided table below.



About 51.6% of the radioactivity was excreted in the urine and 32% in the feces within 48 hours after intravenous dosing in rats. The total radioactivity recovered during the 48-hr period after dosing, including cage washings was 87.4%. The cumulative excretion of the radioactivity in urine and feces of rats after IV administration is given in the sponsor-provided table below.



Single Oral Dosing:

After oral administration of 25, 50 and 100 μg/kg of tritiated RU-0211 to rats, the maximum plasma concentrations were reached within 45 minutes. The C_{max} values increased linearly with increasing doses. There were detectable plasma concentrations at 4 hrs, 6 hrs and 24 hours after dosing of 25, 50 and 100 μg/kg ^{3}H-RU-0211 respectively. The levels of plasma radioactivity at different times after oral administration of 25, 50 and 100 μg/kg doses of ^{3}H-RU-0211 in rats is shown in the sponsor-provided tables below.

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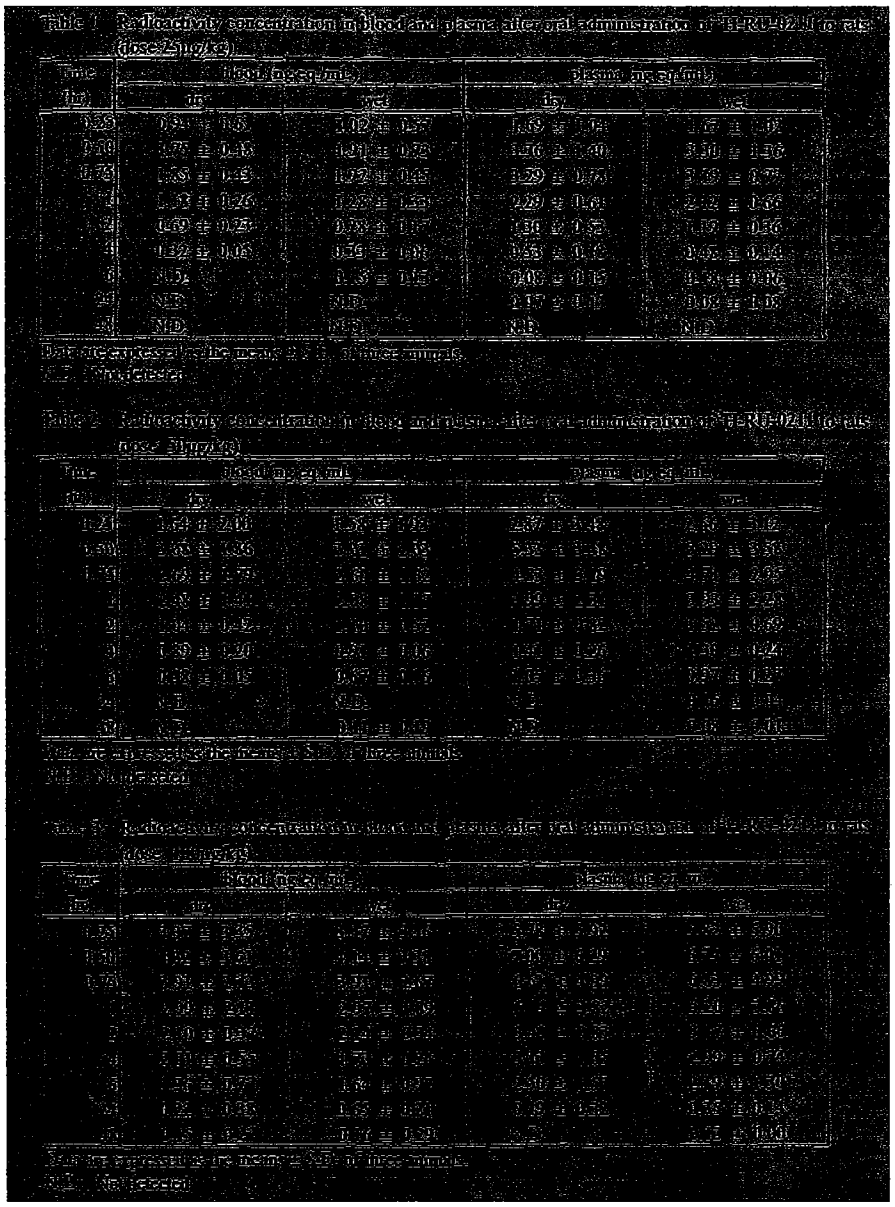
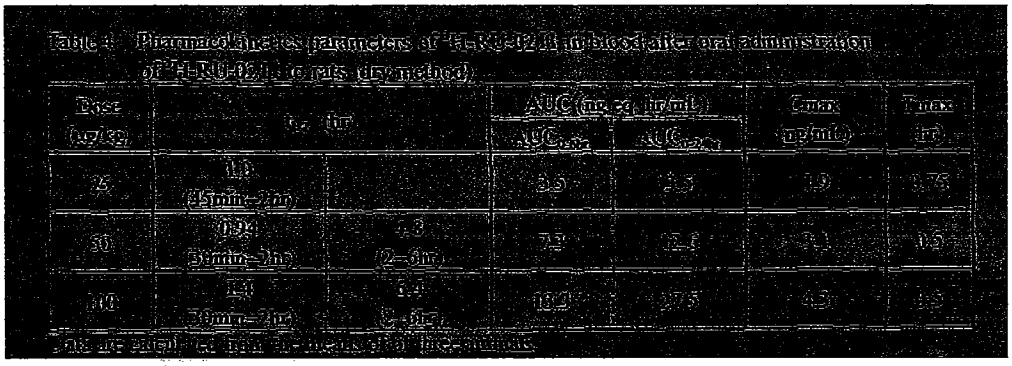
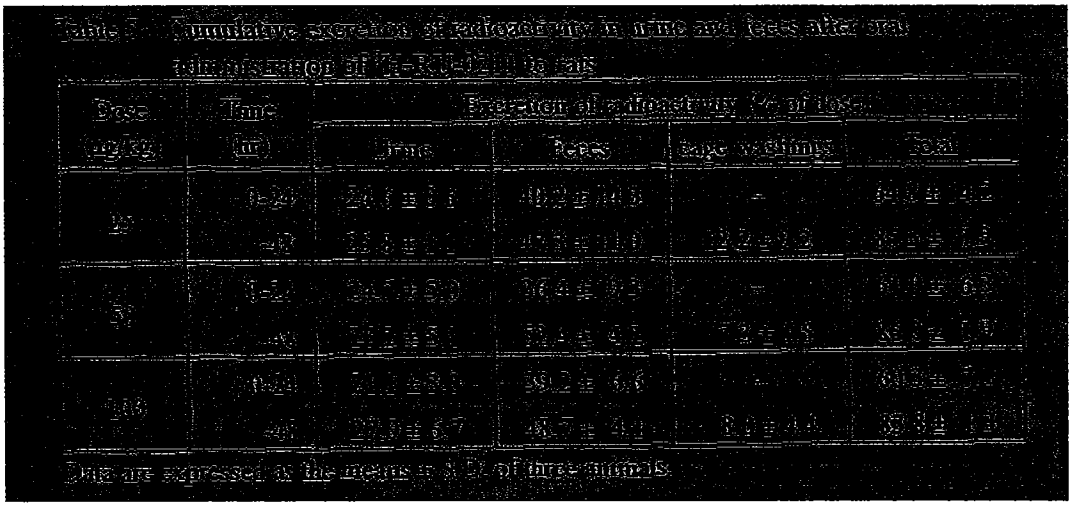


The AUC values also increased dose-dependently with increasing doses; the values ranged from 3.5 to 27.5 ng.eq.hr/ml. The $T_{1/2\alpha}$ values ranged from 0.5 to 2.0 hrs and the $T_{1/2\beta}$ ranged from 2 to 6 hrs. The pharmacokinetic parameters after oral administration of RU-0211 in rats are shown in the sponsor-provided table below.



Following oral administration of 25, 50 and 100 μg/kg of ^{3}H-RU-0211 to rats, 25.8, 26.2 and 27.0% of the radioactivity was excreted in the urine and 47.3, 52.4 and 48.7% in the feces respectively. The total radioactivity excreted, including the case washings, were 85.3, 86 and 83.8% respectively. The cumulative excretion of the radioactivity in the urine and feces after oral administration of 25, 50 and 100 μg/kg doses in rats is given in the sponsor's table below.



Comments and Conclusion: The AUC and C_{max} values after IV dosing of radiolabeled RU-0211 in rats were higher than that achieved by the oral route of the same dose. Thus, there is a possibility of the first-pass metabolism of the drug when given by the oral route. The sponsor used only the male animals for the pharmacokinetic studies; thus, the pharmacokinetic differences between the males and the females, if any, could not be determined.

Pharmacokinetics of RU-0211 after Single Oral Administration to Male and Female Rats (Study # EIH116)

Methods: The pharmacokinetics of ^{3}H-RU-0211 was studied in male and female rats after oral administration of a 50 µg/kg dose. Three male and 3 female - CD Sprague-Dawley rats were orally administered a single 50 µg/kg dose of ^{3}H-RU-0211 (as mini capsules). Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 48 hrs after administration of the dose. Urine and feces were collected at 0-24 hrs and 0-48hrs following administration. Blood and plasma samples were analyzed by both dry and wet methods. In the dry method, aliquots of blood or plasma were dried and then solubilized before measuring the radioactivity. In the wet method, aliquots of solubilized blood samples or plasma were mixed with the scintillator and the radioactivity measured.

Results: Following oral administration of a single dose of 50 µg/kg to rats, the maximum mean plasma radioactivity concentration was reached in approximately 2 hrs in males in approximately 3 hrs in females (measured by the dry method). The C_{max} and AUC values in females were greater than those in males. The elimination half-life ($T_{1/2\beta}$) was approximately 2 hrs in both males and females. Plasma pharmacokinetic parameters in male and female rats are summarized in the Table below.

Pharmacokinetic Parameters of ^{3}H-RU-0211 in Blood and Plasma After Oral Administration to Male and Female Rats*

Sex	Sample	Assay Method	Elimination Half-Life	AUC (ng eq.·hr/mL)		C_{max} (ng eq./mL)	T_{max} (hr)
			($T_{1/2\beta}$) (2-6 hr)	0-8hr	0-24hr		
Male	Blood	Dry	6.9**	10.9 ± 0.6	16.2 ± 1.9	2.36 ± 0.17	1.83 ± 1.88
		Wet	2.1 ± 0.6	10.4 ± 0.5	14.2 ± 1.7	2.19 ± 0.16	1.75 ± 1.95
	Plasma	Dry	2.0 ± 0.4	18.6 ± 0.6	24.7 ± 1.7	4.06 ± 0.18	1.75 ± 1.95
		Wet	1.9 ± 0.2	17.2 ± 0.8	22.3 ± 1.2	3.78 ± 0.29	1.83 ± 1.89
Female	Blood	Dry	4.5 ± 3.0	28.4 ± 2.7	38.9 ± 6.8	6.66 ± 2.47	2.83 ± 2.02
		Wet	5.3 ± 5.7	28.3 ± 3.0	38.4 ± 6.3	6.77 ± 2.87	2.83 ± 2.02
	Plasma	Dry	2.4***	50.3 ± 6.4	67.1 ± 15.0	12.28 ± 5.02	2.83 ± 2.02
		Wet	13.6 ± 3.9	47.5 ± 5.1	62.4 ± 13.2	11.75 ± 4.59	2.92 ± 1.88

*Data are expressed as the means ± SD of parameters calculated from three animals.
**Mean value from two animals
***Value from only one animal

Following oral administration of ^{3}H-RU-0211 to rats, the radioactivity was excreted by both urinary and fecal pathways. In 48 hrs after administration of the dose, 35.1% and 46.8% of the administered radioactivity was excreted in the urine, and 57.8% and 37.2% of the administered radioactivity was excreted in feces of males and females, respectively. In 48 hours after dosing, 94.6% and 88.5% of the radioactivity were recovered in urine, feces and cage washings of males and females, respectively. Cumulative excretion of the radioactivity in urine and feces of male and female rats are summarized in the Table below.

Cumulative Excretion of Radioactivity in Urine and Feces After Oral Administration of ^{3}H-RU-0211 to Male and Female Rats

Sex	Cumulative Time Post Dose (hr)	Cumulative Excretion of Radioactivity (% of Dose)			
		Urine	Feces	Cage Washings	Total
Male	0-24	34.9 ± 5.8	52.8 ± 9.5	–	87.7 ± 9.1
	0-48	35.1 ± 5.9	57.8 ± 6.9	1.6 ± 0.9	94.6 ± 11.7
Female	0-24	46.4 ± 4.7	32.6 ± 10.6	–	79.0 ± 6.1
	0-48	46.8 ± 5.0	37.2 ± 8.7	4.5 ± 1.1	88.5 ± 3.9

Pharmacokinetics of RU-0211 after Oral Administration to Rats and Mice (Study Report Nos. RTU/SR01-068, RTU/SR01-069 and RTU/SR01-070).

Methods: Pharmacokinetic studies of RU-0211 were conducted after oral administration of ^{3}H-RU-0211 to rats (50 µg/kg and 100 µg/kg single doses) and mice (25 µg/kg and 50 µg/kg single doses). ^{3}H-RU-0211 was dissolved in medium chain fatty acid triglyceride ( ) and administered by oral gavage. Samples of blood were collected at 15, 30 and 45 minutes and 1, 2, 4, 6, 8, 24 and 48 hours after administration in rats, and at 5, 10, 15, 30 and 45 minutes and 1, 2, 4, 6, 8, 24 and 48 hours after administration in mice. The blood and plasma radioactivity was measured by liquid scintillation counting. Blood and plasma samples were analyzed by both dry (samples were dried and then dissolved in a solvent before counting) and wet (blood samples were solubilized and decolorized before counting) methods. The radioactivity in the urine and fecal samples and cage washings was also determined.

Results: The maximum plasma radioactivity concentration (C_{max}) and $AUC_{0\text{-}24h}$ values increased with increasing dose in both rats and mice. After administration of 50 and 100 µg/kg doses to rats, the C_{max} values were 8.14 and 13.72 ng eq/ml and the $AUC_{0\text{-}24h}$ values were 35.5 and 76.8 ng eq.hr/ml, respectively dry method). In mice, after 25 and 50 µg/kg doses, the Cmax values were 14.5 and 41.63 ng eq./ml and the $AUC_{0\text{-}24h}$ values were 36.9 and 66.2 ng eq.hr/ml, respectively. After a 50 µg/kg dose, the $T_{½}$ β-value was 2.1 hours in rats and 2.2 hours in mice. The pharmacokinetic parameters in rats and mice after single oral doses of ^{3}H-RU-0211 are summarized in the Table below.

Table: Plasma pharmacokinetic parameters of RU-0211 in rats and mice after administration of single oral doses.

Dose (µg/kg)	Assay method	$T_{1/2}$ (Hour)		$AUC_{0\text{-}24h}$ (ng eq/hr/ml)	C_{max} (ng eq./ml)	T_{max} (min)
Rat		α-Phase	β-Phase			
50	Dry	--	2.1±0.6	35.5±12.8	8.14±2.87	1.1±0.4
	Wet	--	2.2±1.0	33.5±11.9	7.92±3.00	1.0±0.5
100	Dry	--	3.5±3.1	76.8±49.6	13.72±1.52	0.33±0.14
	Wet	--	14.2±9.2	72.8±41.8	12.59±2.06	0.33±0.14
Mouse						
25	Dry	0.4	2.8	36.9	14.5	5
	Wet	0.4	2.9	42.5	15.28	15
50	Dry	0.3	2.2	66.2	41.63	10
	Wet	0.3	2.5	74.4	41.8	10

After oral dosing, the elimination of the radioactivity was via the urine and feces. In mice, the urinary route of elimination was predominant, while in rats no apparent differences were observed between the urinary and fecal excretion. The cumulative excretion of the radioactivity in urine and feces after oral administration of RU-0211 to rats and mice are shown in the Table below.

Table: cumulative excretion of the radioactivity in urine and feces after oral administration of RU-0211 to rats and mice

Dose (µg/kg)	Time (hour)	Cumulative Excretion (Percent of dose)			
Rat		Urine	Feces	Cage washings	Total
50	0-24	37.1±8.8	37.3±6.5	--	74.3±8.2
	0-48	37.5±9.0	42.5±4.6	8.0±5.4	88.0±3.7
100	0-24	35.3±4.4	28.1±4.1	--	63.4±5.9
	0-48	36.2±4.8	37.6±5.8	10.2±0.2	84.0±2.2
Mouse					
25	0-24	63.7±15.8	11.3±3.1	11.8±6.5	86.8±9.8
	0-48	57.6±9.3	17.9±6.3	18.7±5.7	94.3±9.4
50	0-24	49.7±28.1	16.0±2.9	14.6±6.3	80.4±21.8
	0-48	51.5±22.4	16.8±3.7	23.5±13.3	91.9±12.6

Pharmacokinetics of RU-0211 after Single Oral Administration to Mice (Study Report #SPI/SR03-025; Study # EIH108)

Methods: The pharmacokinetics of ^{3}H-RU-0211 was studied in mice after oral administration of a 100 µg/kg dose. Male B6C3F1 mice were administered a 100 µg/kg dose of ^{3}H-RU-0211 (6.51 mCi/mg) by oral gavage (1 ml/kg). Blood samples were collected at 15, 30 and 45 min, and 1, 2, 4, 6, 8, 24 and 48 hrs after administration of the dose (3 animals/time-point). Urine and feces were collected for 0-24 hrs and 0-48 hrs post-dosing periods from 3 animals designated for blood collection at these respective time points. Cage radioactivity was also collected at 24 hrs and 48 hrs after dosing by washing the cages with water. Radioactivities in blood and plasma samples were analyzed by both dry and wet methods. In the dry method, aliquots of blood or plasma were dried and then solubilized before measuring the radioactivity. In the wet method, aliquots of solubilized blood samples or plasma were mixed with the scintillator and the radioactivity measured.

Results: Following oral administration of a 100 µg/kg dose of ^{3}H-RU-0211 to mice, the mean plasma radioactivity concentration reached at maximum levels at 15 minutes after dosing. Based on the determination of the radioactivity by the dry method, the C_{max}, $T_{1/2a}$, $T_{1/2\beta}$ and $AUC_{0\text{-}24hr}$ values were 75.37 ng eq/ml, 1.1 hrs, 10.8 hrs and 219.5 ng eq.hr/ml, respectively. When measured by the wet method, the plasma pharmacokinetic parameters were similar to those observed by the dry method. Pharmacokinetic parameters of ^{3}H-RU-0211 in blood and plasma following oral administration to mice are shown in the Table below.

Pharmacokinetic Parameters of ^{3}H-RU-0211 in Blood and Plasma after Oral Administration to Mice*

Dose (µg/kg)	Sample	Assay Method	$T_{1/2}$ (hr)		AUC (ng eq.·hr/mL)		C_{max} (ng eq./mL)	T_{max} (min)
			Distribution ($T_{1/2\alpha}$) (1 - 4 hrs)	Elimination ($T_{1/2\beta}$) (8 – 48 hrs)	0-8hr	0-24hr		
100	Blood	Dry	1.1	8.9	110.0	135.3	45.01	15
		Wet	1.2	17.2	118.7	160.2	45.86	15
	Plasma	Dry	1.1	10.8	182.1	219.5	75.37	15
		Wet	1.2	15.2	189.5	237.9	74.38	15

* PK parameters were calculated based on the mean radioactivity values obtained from three separate mice at each postdosing time point.

The radioactivity was predominantly excreted via the urine. Within 48 hrs of administration of the radiolabeled compound to the animals, 55.7% of the administered radioactivity was excreted in the urine, and 26.2% of the radioactivity was excreted in feces. Over 85% of the administered radioactivity (including cage wash) was recovered in 48 hours following administration of the dose. Cumulative excretion of the radioactivity in the urine and feces of mice is summarized in the Table below.

Cumulative Excretion of Radioactivity in Urine and Feces after Oral Administration of ^{3}H-RU-0211 to Mice*

Dose (µg/kg)	Cumulative Time Post Dose (hr)	Cumulative Radioactivity (% of Dose)			
		Urine	Feces	Cage Washings	Total
100	0-24	46.3 ± 3.6	29.4 ± 4.7	5.6 ± 2.3	81.2 ± 3.5
	0-48	55.7 ± 5.3	26.2 ± 4.7	3.9 ± 2.4	85.9 ± 4.9

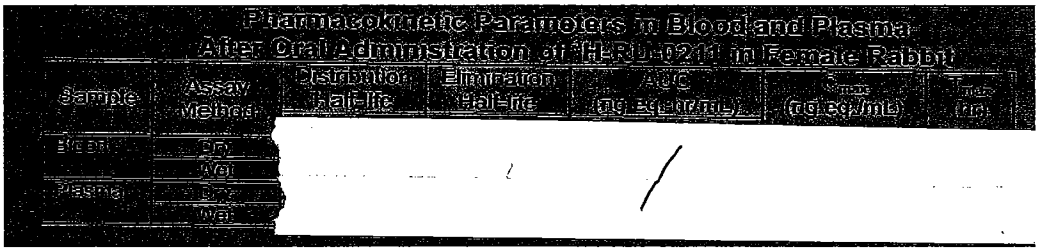
*Data are expressed as the mean ± SD of values obtained from three animals bled for PK sampling at the 24 hr or 48 hr time points.

Pharmacokinetics of RU-0211 after Single Oral Administration in Female Rabbit (Study Report #SPI/SR05-011; Study # EIH118-1)

Methods: The pharmacokinetics of ^{3}H-RU-0211 was studied in a female rabbit following oral administration of a 5 µg/kg dose. A single female NZW rabbit was administered an oral dose of 5 µg/kg ^{3}H-RU-0211 (39.45 mCi/mg). Samples of blood were collected at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours after administration of the dose. Urine and feces were collected at 0-24 and 24-48 hrs post-dosing periods. Blood and plasma samples were analyzed by both dry and wet methods. In the dry method, an aliquot of the sample was dried and solubilized before measuring the radioactivity. In the wet method, each blood sample was solubilized, and the radioactivity was measured after mixing with the scintillator.

Results: Based on the plasma radioactivity concentrations measured by the dry method, the T_{max}, C_{max},

$T_{1/2a}$, $T_{1/2\beta}$ and $AUC_{0\text{-}24hr}$ values were 5.0 hrs, 1.05 ng eq/ml, 2.4 hrs, 9.1 hrs and 7.4 ng eq.hr/ml, respectively. The radioactivity concentrations and PK values obtained by the wet method were similar to those of the dry method. Unchanged ^{3}H-RU-0211 was detected in the plasma samples by HPLC analysis. In addition, several major metabolites with higher polarity were also detected at both 1 and 5 hours after dosing. The blood and plasma pharmacokinetic parameters for the radioactivity after oral administration of a 5 µg/kg dose to the rabbit are shown in the Table below.



In 48 hrs following administration, 86.9% of the radioactivity was excreted in the urine. After including cage washings, 88.2% of the administered radioactivity was excreted in 48 hrs after oral administration of ^{3}H-RU-0211. The excretion profiles of the radioactivity in urine and feces are shown in the Table below.

Cumulative Excretion of Radioactivity in Urine and Feces After Oral Administration of ^{3}H-RU-0211 to Female Rabbit

Cumulative Time Post Dose (hour)	Cumulative Excretion of Radioactivity (% of Dose)			
	Urine	Feces	Cage Washings	Total
0-24	77.8	0.4	–	78.1
0-48	86.9	0.6	0.6	88.2

Pharmacokinetics of RU-0211 after Oral Administration to Monkey (Study Report #RTU/SR01-065; Study # EIH106):

Methods: The pharmacokinetics of ^{3}H-RU-0211 was studied in a monkey after oral administration of a 5 µg/kg dose. A single male cynomolgus monkey was used in the study, and a single 5 µg/kg dose of ^{3}H-RU-0211 (specific activity, 241 MBq/mg) was orally administered as a capsule after overnight fasting. Blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 24 and 48 hours after administration. Urine and fecal samples were collected at 0-8 hrs, 8-24 hrs and 24-48 hrs after dosing. The radioactivity in all samples was measured with a liquid scintillation counter. Blood and plasma samples were analyzed by both dry and wet methods. In the dry method, an aliquot of the sample was dried and solubilized before measuring the radioactivity. In the wet method, each blood sample was solubilized, and the radioactivity was measured after mixing with the scintillator.

Results: Following oral administration of a 5 µg/kg dose of RU-0211 to monkeys, plasma radioactivity concentrations increased sharply and reached a maximum level at 4 hours after dosing. After 4 hrs, the radioactivity concentrations declined with time. Based on the plasma radioactivity concentrations

measured by the dry method, the C_{max} value, distribution half-life ($T_{1/2a}$), the elimination half life ($T_{1/2ß}$) and the AUC 0-24h were 1.37 ng eq/ml, 0.7 hrs, 8.9 hrs and 6.4 ng eq.hr/ml, respectively. When measured by the wet method, radioactivity concentrations were generally similar to those obtained by the dry method; however, the elimination half-life was longer when measured by the wet method. The radioactivity in the blood was somewhat lower than that of plasma, when measured by the dry or wet method. The pharmacokinetic parameters in blood and plasma of the monkey are shown in the Table below.

Pharmacokinetic Parameters of ^{3}H-RU-0211 in Blood and Plasma after Oral Administration to Monkey

Dose (µg/kg)	Sample	Assay Method	$T_{1/2}$ (hr)		AUC (ng eq.·hr/mL)		C_{max} (ng eq./mL)	T_{max} (hr)
			Distribution ($T_{1/2\alpha}$)	Elimination ($T_{1/2\beta}$)	0-8hr	0-24hr		
5	Blood	Dry	0.9	12.3	3.4	4.3	0.83	4
		Wet	0.9	39.0	3.8	5.9	0.96	4
	Plasma	Dry	0.7	8.9	5.3	6.4	1.37	4
		Wet	0.8	46.7	5.4	7.7	1.38	4

Within 48 hours of administration of a 5 µg/kg dose to monkeys, 72.5% of the administered radioactivity was excreted in the urine and 0.039% excreted in the feces. Cumulative excretion of the radioactivity in urine and feces is shown in the Table below.

Cumulative Excretion of Radioactivity in Urine and Feces after Oral Administration of ^{3}H-RU-0211 to Monkey

Dose (µg/kg)	Cumulative Time Post Dose (hr)	Cumulative Radioactivity (% of Dose)		
		Urine	Feces	Total
5	0-8	55.6	-	55.6
	0-24	68.0	0.012	68.0
	0-48	72.5	0.039	72.6

Pharmacokinetics of RU-0211 after Single Oral Administration to Dog (Study Report #RTU/SR01-066; Study # EIH107)

Methods: The pharmacokinetics of ^{3}H-RU-0211 was studied in a dog following oral administration of a 5 µg/kg dose. A single male beagle dog was administered a 5 µg/kg oral dose (capsule) of ^{3}H-RU-0211 following overnight fasting. Blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 24, 48 and 72 hrs after administration of the dose. Urine and feces were collected at 0-24 hrs, 24-48 hrs and 48-72 hrs post-dose. Blood and plasma samples were analyzed by both dry and wet methods. In the dry method, an aliquot of the sample was dried and solubilized before measuring the radioactivity. In the wet method, each blood sample was solubilized, and the radioactivity was measured after mixing with the scintillator.

Results: Following oral administration of a 5 µg/kg dose of ^{3}H-RU-0211 to dogs, the maximum plasma and blood radioactivity concentrations were reached in 3 hrs after dosing, and then declined with time. Based on the plasma radioactivity concentrations measured by the dry method, the C_{max} value, distribution half-life ($T_{1/2a}$), the elimination half life ($T_{1/2\beta}$) and the AUC 0-24h were 3.48 ng eq/ml, 1.5 hrs, 12.8 hrs and 15.0 ng eq.hr/ml, respectively. When measured by the wet method, radioactivity concentrations were generally similar to those obtained by the dry method. The radioactivity in the blood was slightly lower than that of plasma, when measured by the dry or wet method. The pharmacokinetic parameters in blood and plasma of the dog after oral administration of a 5 µg/kg dose of ^{3}H-RU-0211 are shown in the Table below.

Pharmacokinetic Parameters of ^{3}H-RU-0211 in Blood and Plasma after Oral Administration to Dog

Dose (µg/kg)	Sample	Assay Method	$T_{1/2}$ (hr)		AUC (ng eq.·hr/mL)		C_{max} (ng eq./mL)	T_{max} (hr)
			Distribution ($T_{1/2\alpha}$)	Elimination ($T_{1/2\beta}$)	0-8hr	0-24hr		
5	Blood	Dry						
		Wet						
	Plasma	Dry						
		Wet						

Within 72 hours after administration of a 5 µg/kg dose of ^{3}H-RU-0211 to dogs, 70.3% of the administered radioactivity was excreted in the urine and 14.4% of the radioactivity was excreted in the feces. Total radioactivity recovered in 72 hrs, including cage washing, was 85.4%. Cumulative excretion of the radioactivity in urine and feces is shown in the Table below.

Cumulative Excretion of Radioactivity in Urine and Feces after Oral Administration of ^{3}H-RU-0211 to Dog

Dose (µg/kg)	Cumulative Time Post Dose (hr)	Cumulative Radioactivity (% of Dose)			
		Urine	Feces	Cage Washings	Total
5	0-24	65.9	0.035	–	66.0
	0-48	70.1	13.8	–	83.8
	0-72	70.3	14.4	0.7	85.4

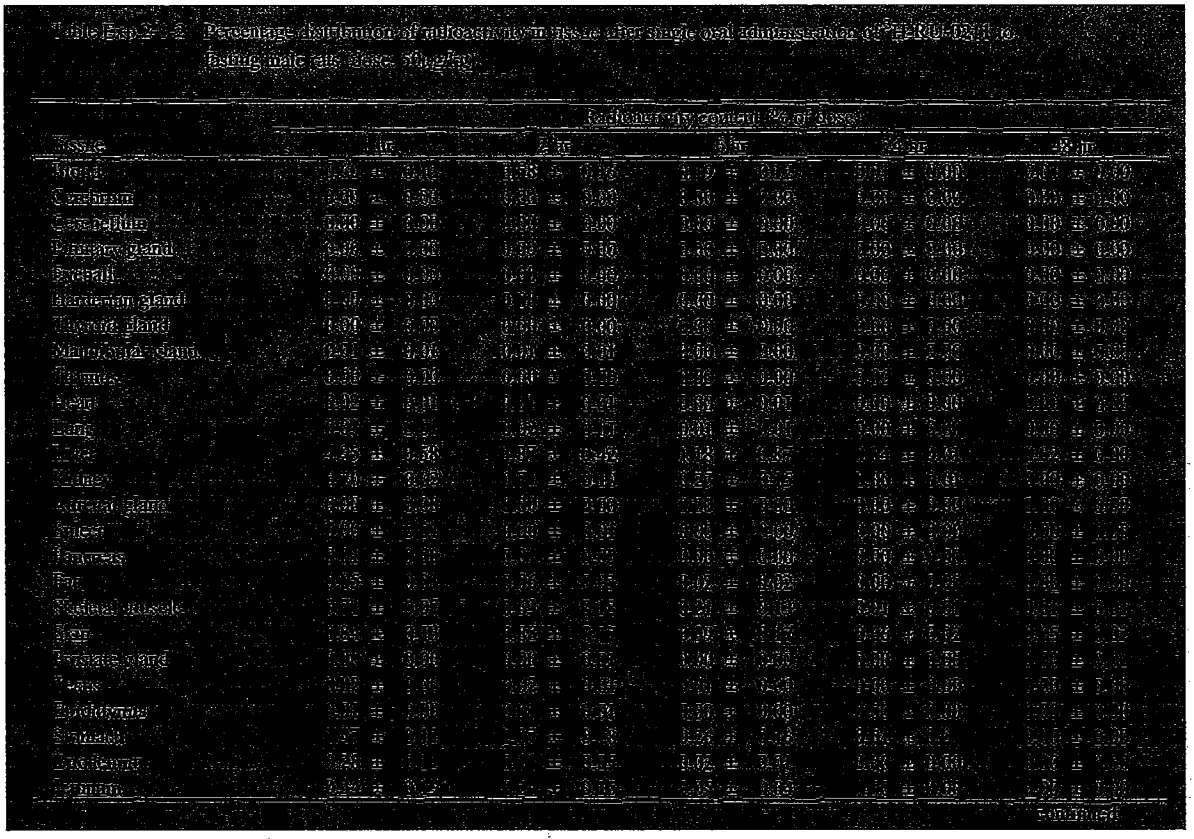
2.6.4.4 Distribution

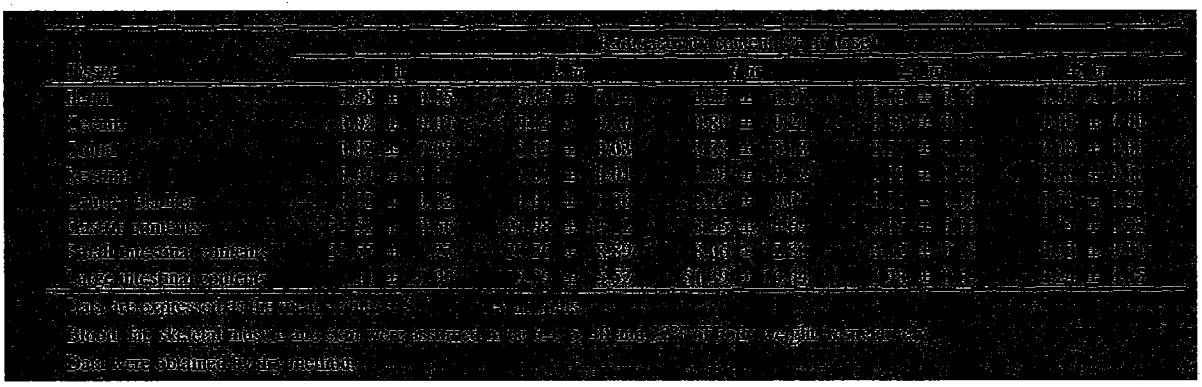
Tissue Distribution of Radioactivity in Rats after Single Oral Administration of ^{3}H-RU-0211 (Study Report No. RTU/SR01-080).

Methods: The tissue distribution of the radioactivity in male and female Sprague Dawley rats was studied after oral administration of a 50 µg/kg dose of ^{3}H-RU-0211. The animals were sacrificed at

different times after administration of the dose and the radioactivity in different tissues was measured by liquid scintillation counting.

Results: One hour after administration of ^{3}H-RU-0211 to rats, the radioactivity concentrations were highest in the stomach and duodenum (20.5 and 14.9 times higher than the plasma concentration of 8.26 ng eq./ml). After 6 hours, the peak concentrations were detected in the cecum, colon and rectum; in other tissues, the peak was reached in 1-2 hours. The next higher concentrations were detected in the liver, urinary bladder, jejunum, kidney and ileum (4.7 to 9.7 times higher than plasma). Elimination of the radioactivity was rapid; the radioactivity in most tissues (except cecum, colon and rectum) decreased by 50% to 94% in 6 hours. After 48 hours, the radioactivity in all tissues was decreased by 83%. The radioactivity in the blood cells was not more than 5.7% of the dose, up to 6 hours after administration. The tissue radioactivity concentrations in the females were slightly higher than that of males at the early time points of determination. The distribution of radioactivity in male and female rats after administration of a single dose of 50 µg/kg ^{3}H-RU-0211 is shown in the sponsor's Tables below.





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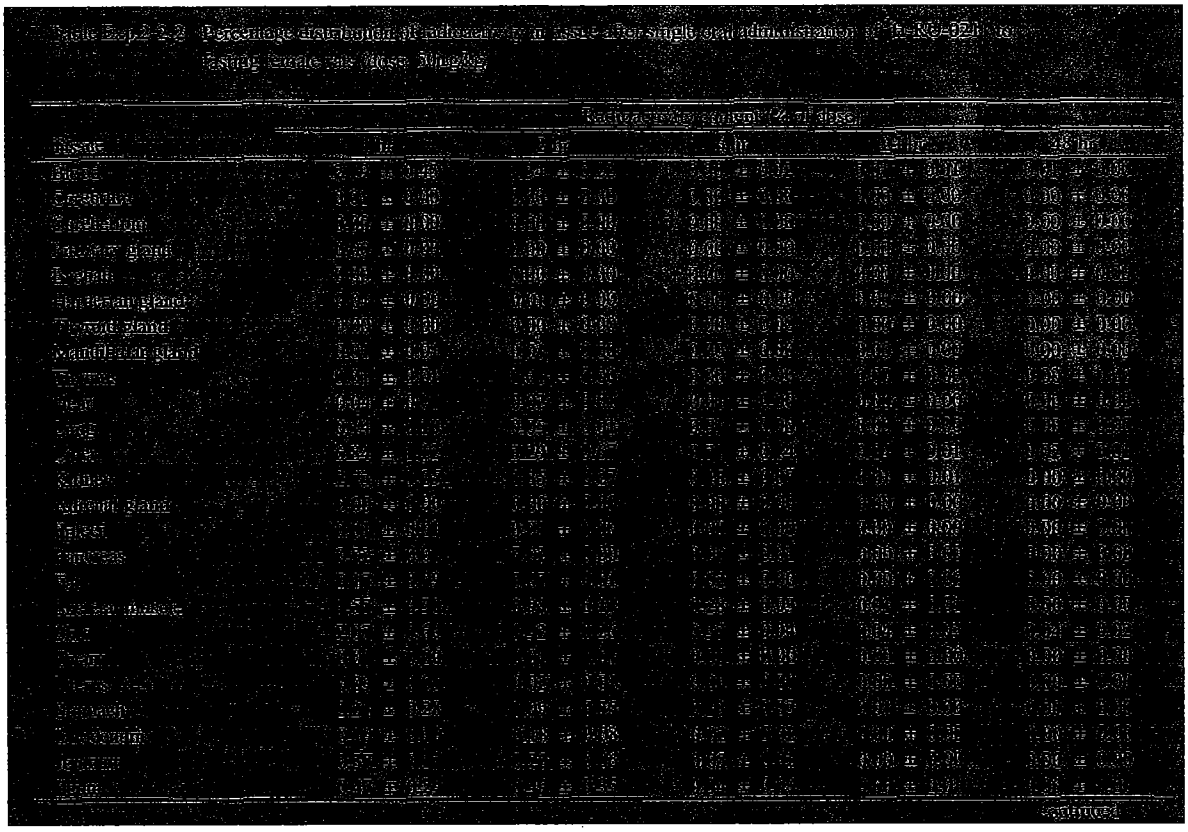
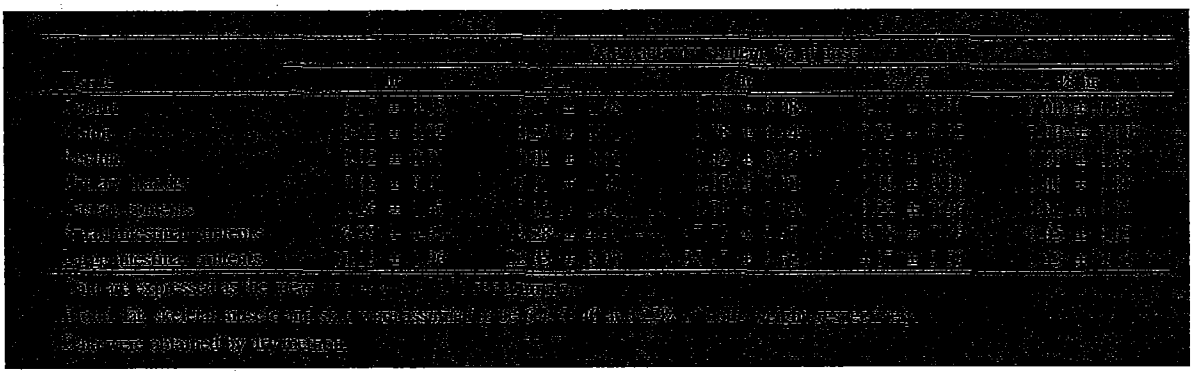




Plasma Protein Binding of RU-0211 in the Rat, Dog, Mouse and Human Samples.

Methods: *In vitro* plasma protein binding of ^{3}H-RU-0211 was determined using the male and female rat, mouse, dog and human plasma. In vivo plasma protein binding was examined in rats after oral administration of ^{3}H-RU-0211 to rats. The plasma samples were incubated with radiolabeled RU-0211 at 1, 10 and 30 ng/ml. In the *in vivo* study, rats received ^{3}H-RU-0211 (50 µg/kg) by the oral route and samples of blood were collected at 1, 2 and 6 hours after the dosing. The bound and unbound radioactivities were separated by centrifugation in a ' — device and the radioactivity counted. The reversibility of the protein binding was examined after methanol

extraction of the plasma samples (at 30 ng/ml of RU) and determining the radioactivity in the extracts.

Results: The *in vitro* plasma protein binding of RU-0211 were 94.4-94.7% in the male rat plasma, 89.3-91.1% in the male mouse plasma and 96.3-96.4% in the male dog plasma. From the plasma samples, about 99% of the radioactivity was extractable with methanol. The plasma protein binding in females was comparable to that of males at 1, 10 and 30 ng/ml. After administration of a single oral dose of ^{3}H-RU-0211 to male rats, the plasma protein binding was 46.1%, 54.9% and 61.1% at 1, 2 and 6 hours after administration, respectively. From the plasma samples at 1, 2 and 6 hours, 89.9%, 82.3% and 73.0% of the radioactivity were extractable with methanol, respectively. The *in vitro* and *in vivo* plasma protein binding of RU-0211 is summarized in the Tables below.

Table: In vitro plasma protein binding of ^{3}H-RU-0211 in male and female mouse, rat, dog and human plasma (% binding).

Concentration (ng/ml)	Mouse	Rat	Dog	Human
Male				
30	89.3±0.0	91.9±1.0	96.3±0.3	94.7±0.5
10	90.5±0.6	92.3±0.8	96.4±0.3	94.4±0.7
1	91.1±0.4	93.9±0.9	N.C.	94.6±0.6
Female				
30	92.4±0.6	94.5±0.9	95.6±0.2	94.9±0.5
10	93.2±0.6	94.7±0.1	95.9±0.3	94.4±0.5
1	93.2±1.6	94.9±1.5	96.0 (n=2)	N.C.

Mean ± S.D.; N.C., not calculated.

Table: In vivo plasma protein binding of RU-0211 in male rats (50 µg/kg) and humans (72 µg/kg) after a single oral dose (% binding).

Time (hrs)	% Bound
Rat	
1	46.1
2	54.9
6	61.1
Human	
1	53.7
2	34.5
4	17.2

Thus, the *in vitro* plasma protein binding in dogs was slightly higher than that in humans, rats and mice, while the binding in humans were slightly higher than that in rats and mice. The *in vivo* plasma protein binding in rats and dogs were lower than that *in vitro*. This may be due to rapid

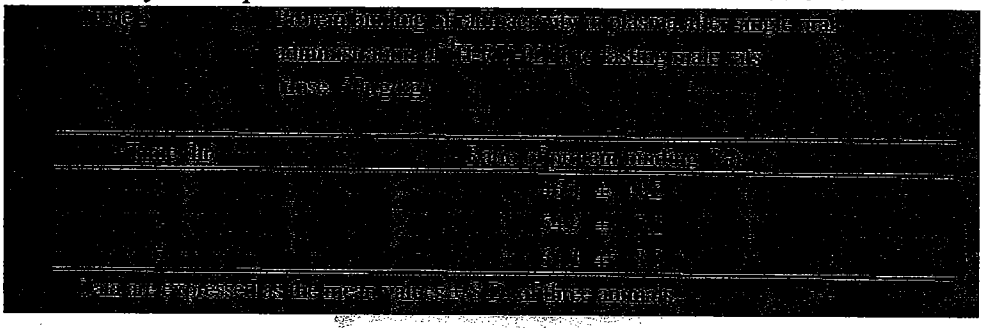
metabolism of the compound after oral administration. The sponsor did not conduct plasma protein binding studies with any of its metabolites.

Plasma Protein Binding in Rats after Oral Administration of ^{3}H-RU-0211 (Study Report # SPI/SR02-022)

Methods: The plasma protein binding of the ^{3}H-RU-0211-derived radioactivity was studied in male rats following oral administration of ^{3}H-RU-0211. Male Sprague-Dawley rats were administered a 50 µg/kg dose of ^{3}H-RU-0211 (7.32 MBq/kg; purity, — by oral gavage. Plasma protein binding ratios were determined using plasma collected at 1, 2 and 6 hrs after dosing (3 animals/time-point), and plasma samples spiked with ^{3}H-RU-0211. Reversibility of the binding was investigated using plasma samples at all sampling times. Bound and unbound radioactivities were separated by an ultrafiltration method. Binding ratio was calculated using the following equation.

Reversibility of binding was determined by methanol extraction of the radioactivity.

Results: Following oral administration of a 50 µg/kg dose of ^{3}H-RU-0211 to male rats, the *in vivo* plasma protein binding ratios were 46.1%, 45.9% and 61.1% at 1, 2 and 6 hr, respectively. From the plasma samples at all sampling times, 89.9%, 82.3% and 73.0% of the radioactivity was extracted with methanol at 1, 2 and 5 hr after administration, respectively. Thus, the ratio radioactivity, unextractable by methanol, increased with time. The *in vivo* protein binding of the radioactivity in the plasma of male rats is shown in the Table below.



2.6.4.5 Metabolism

Metabolites of RU-0211 after Oral Administration to Rat, Dog, Monkey and Mouse (Study Report # SPI/SR05-012; Cross reference# EIH103-4).

Methods: The metabolic profiles were examined in the rat, dog, monkey and mouse after oral administration ^{3}H-RU-0211 (3.96 mCi/mg; radiochemical purity, > — at 50, 5, 5 and 50 µg/kg single doses, respectively. Blood samples were taken at 5, 10, 15, 30 and 45 minutes, and 6 hours after the dosing in rats, 15, 30 and 45 minutes and 1.5, 2, 3, 4, 5, 6, 8, 24 and 48 hours after dosing

in dogs, 15, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, 24 and 48 hours after dosing in monkeys, and 5, 10, 15, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, 24 and 48 hours after dosing in mice. Radioactivity in all samples was measured by liquid scintillation counting. The identity of the metabolites present in plasma, urine and feces were carried out by comparison with reference standards using HPLC analyses. Samples with highest radioactivity at the specified sampling times were selected for metabolic profiling. These included: 5, 10, 15 and 30 min plasma samples, 0-6 hrs urine sample and 0-24 hr fecal sample in rats; 3 hrs plasma sample, 0-24 hr urine sample and 24-48 hrs fecal samples in dogs; 1.5 and 4 hrs plasma samples, 0-8 hrs urine samples 48-144 hrs fecal samples in monkeys; 5, 10, 15 min and 1 hr plasma samples for mice.

Results: In the rat plasma, unchanged RU-0211 was detected at 5, 10 and 15 minutes after dosing, but no unchanged drug was detected at 30 minutes after dosing. In the urine and feces of rats, the unchanged drug was not detected at any time. The unchanged drug was also not detected in dogs and monkeys. In mice the unchanged drug was detected in the plasma for up to 1 hr after dosing. Several metabolite peaks were identified by HPLC in all four species. Among them, 15-hydroxy-RU-0211 (peak C) was the least polar metabolite and the other two relatively more polar metabolites, identified as 2, 3, 4, 5-tetranor-RU-0211 (peak D) and 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 (peak J), were identified in all species. The metabolites were quantitatively different in different species, for example, the most polar metabolite, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-02 was present in highest concentrations in the plasma of mice (5.88 ng eq/ml), followed by dogs (0.31 ng eq/ml) and rats (0.03 ng eq/ml). In the monkey, it was not detected in the plasma, although present in the urine and feces. Thus, it appears that the rate of metabolism of RU-0211 varies in different animal species. The metabolites detected in rat, dog, monkey and mouse plasma are shown in the Tables below.

Metabolites in Rat Plasma after Administration of 50 µg/kg of ^{3}H-RU-0211

Metabolite	Retention Time (min)	Percent of metabolite peaks (Concentration in ng eq./mL)			
		5 mins	10 mins	15 mins	30 mins
Radioactivity concentration (ng eq./mL)		3.47	3.41	3.61	3.45
RU-0211	45	0.73 (0.03)	0.52 (0.02)	0.84 (0.04)	ND (ND)
A	41	0.76 (0.03)	ND (ND)	1.44 (0.06)	ND (ND)
B	36	ND (ND)	0.52 (0.02)	ND (ND)	ND (ND)
C	33	5.63 (0.22)	2.35 (0.08)	2.71 (0.12)	0.89 (0.03)
D	30	12.24 (0.48)	4.73 (0.16)	13.31 (0.59)	12.08 (0.38)
E	22	7.69 (0.30)	1.25 (0.04)	7.58 (0.34)	8.79 (0.27)
F	19	5.83 (0.23)	ND (ND)	7.94 (0.35)	9.52 (0.30)
G	17	0.69 (0.03)	ND (ND)	2.46 (0.11)	0.67 (0.02)
H	13	8.27 (0.33)	3.80 (0.13)	24.19 (1.08)	23.11 (0.72)
I	10	ND (ND)	ND (ND)	1.25 (0.06)	1.09 (0.03)
J	8	28.98 (1.14)	60.07 (2.02)	24.06 (1.07)	31.44 (0.98)
K	6	ND (ND)	3.69 (0.12)	ND (ND)	ND (ND)
L	4	ND (ND)	ND (ND)	ND (ND)	ND (ND)
M	3	1.83 (0.07)	ND (ND)	1.17 (0.05)	0.71 (0.02)

ND: Not detected

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Metabolites in Dog and Monkey Plasma after Administration of 5 µg/kg of ^{3}H-RU-0211

Metabolite	Retention Time (min)	Percent of metabolite peaks (Concentration in ng eq./mL)		
		Dog	Monkey	
		3 hrs	1.5 hrs	4 hrs
Radioactivity concentration (ng eq./mL)		2.82	0.87	1.12
RU-0211	45	ND (ND)	ND (ND)	ND (ND)
A	41	5.10 (0.17)	ND (ND)	ND (ND)
B	36	1.87 (0.06)	ND (ND)	ND (ND)
C	33	3.50 (0.12)	ND (ND)	ND (ND)
D	30	25.42 (0.87)	2.13 (0.02)	ND (ND)
E	22	9.12 (0.31)	ND (ND)	ND (ND)
F	19	5.38 (0.18)	23.09 (0.24)	13.36 (0.14)
G	17	0.65 (0.02)	ND (ND)	ND (ND)
H	13	5.57 (0.19)	14.01 (0.15)	13.78 (0.14)
I	10	ND (ND)	ND (ND)	ND (ND)
J	8	9.02 (0.31)	ND (ND)	ND (ND)
K	6	ND (ND)	14.77 (0.15)	33.37 (0.34)
L	4	ND (ND)	ND (ND)	ND (ND)
M	3	3.70 (0.13)	9.84 (0.10)	6.63 (0.07)

ND: Not detected

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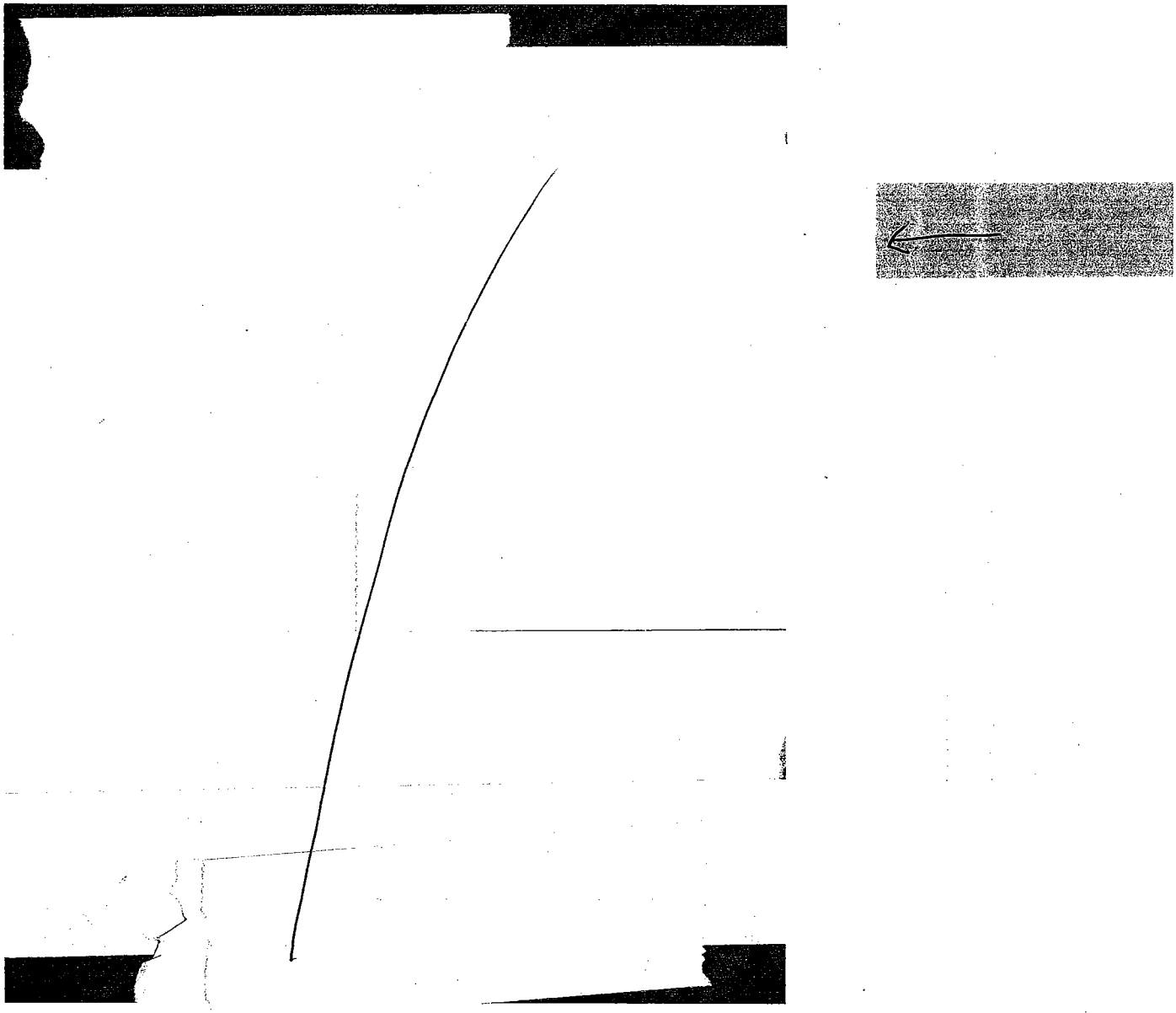
ON ORIGINAL

Metabolites in Mouse Plasma after Administration of 50 µg/kg of ^{3}H-RU-0211

Metabolite	Retention time (min)	Percent of metabolite peaks (Concentration in ng eq./mL)			
		5 mins	10 mins	15 mins	1 hr
Radioactivity concentration (ng eq./mL)		19.02	42.03	31.36	17.93
RU-0211	45	0.94 (0.17)	0.48 (0.21)	0.68 (0.22)	2.18 (0.41)
A	41	2.26 (0.42)	1.46 (0.64)	1.39 (0.46)	0.34 (0.06)
B	36	0.13 (0.02)	0.09 (0.04)	0.50 (0.17)	0.63 (0.12)
C	33	2.08 (0.38)	1.06 (0.47)	1.01 (0.33)	0.67 (0.13)
D	30	18.92 (3.49)	10.54 (4.64)	7.57 (2.48)	4.31 (0.82)
E	22	27.94 (5.15)	15.24 (6.71)	12.91 (4.23)	7.76 (1.47)
F	19	3.34 (0.62)	3.92 (1.73)	3.58 (1.17)	3.39 (0.64)
G	17	6.28 (1.16)	8.95 (3.94)	10.48 (3.44)	8.12 (1.54)
H	13	3.38 (0.62)	2.12 (0.93)	4.47 (1.47)	2.02 (0.38)
I	10	0.15 (0.03)	4.22 (1.86)	1.29 (0.42)	3.25 (0.61)
J	8	22.32 (4.11)	38.97 (17.16)	35.16 (11.53)	31.06 (5.88)
K	6	1.09 (0.20)	4.47 (1.97)	6.52 (2.14)	15.12 (2.86)
L	4	ND (ND)	0.65 (0.28)	1.90 (0.62)	5.78 (1.10)
M	3	ND (ND)	1.12 (0.49)	1.48 (0.49)	4.45 (0.84)

ND: Not detected

The proposed metabolic pathway for RU-0211 is shown in the sponsor's Figure below.



In Vitro Metabolism of RU-0211 –Comparative Metabolism Study using Rat, Dog and Human Liver Microsomes (Study Report # RTU/SR01-077)

Methods: To compare the species and sex differences in the *in vitro* metabolism of RU-0211, the metabolic profiles of ^{3}H-RU-0211 were determined after incubating the compound with rat, dog and human liver microsomes. ^{3}H-RU-0211 (radiochemical purity —, admixed with unlabeled RU-0211 (10, 100 and 1000 ng/ml, final concentration) was incubated in phosphate buffer containing magnesium chloride and NADP generating system with the liver microsomes (0.5 mg

protein/ml) at 37^{0}C for different time periods. The metabolic profiles were determined by HPLC analyses by comparison with reference standards.

Results: When RU-0211 was incubated with human liver microsomes, the main metabolite detected was 15-hydroxy-RU-0211. At 100 ng/ml RU concentration, the percentage of this metabolite were 53.3%, 83.3%, 86.5% and 83.7% after 0, 15, 30 and 60 minutes of incubation, respectively; at 1000 ng/ml, the percentage of the metabolite were 42.7%, 75.1%, 85.8% and 84%, respectively. In addition, another peak, identified as — and considered as a degradation product, was also identified in all samples — or less).

In the rat liver microsomes, the percentage of degradation of RU-0211 (1000 ng/ml; 15 minutes) was 23.2% in males and 10.9% in females. The formation of the main metabolite, 15-hydroxy-RU-0211, was 18.6% in males and 7.4% in females. In the dog liver, the percentage of degradation was 38.1% and 49.5% and the percentage of 15-hydroxy-RU-0211 was 37.4% and 49.4% in males and females, respectively. In humans, no sex differences in the metabolism were observed; 61.8% and 60.2% degradation was observed in males and females, respectively. The percent formation of 15-hydroxy-RU-0211 was 62.7% and 60.8% in males and females, respectively. The percent degradation of RU-0211 and the percent formation of the metabolite in rat dog and human are summarized in the Table below.

Table: In vitro metabolism of ^{3}H-RU-0211 by rat, dog and human liver microsomes (% of control).

Metabolite	Rat		Dog		Human	
	Male	Female	Male	Female	Male	Female
^{3}H-RU-0311 (% degradation)	23.2	10.9	38.1	49.5	61.8	60.2
15-hydroxy-RU-0211 (% formation)	18.6	7.4	37.4	49.4	62.7	60.8

Thus, in the rat, dog and human liver microsomes, RU-0211 was metabolized to 15-hydroxy-RU-0211, and there was a sex and species differences in the metabolism. The metabolism was faster in male rats as compared with the females and in female dogs as compared with the males. The metabolism of RU-0211 was faster in the human liver, followed by dog and rat.

Metabolism in Gastrointestinal Tissues after Single Oral Administration of RU-0211 to Rats (preliminary study): Study # EIH119

Methods: The metabolism of RU-0211 in the plasma and gastrointestinal tissues of male Sprague-Dawley rats was examined after oral administration of a 50 µg/kg dose of ^{3}H-RU-0211 (39.45 mCi/mg; radiochemical purity ≥ —. Blood samples were collected at 5, 10, 15 and 30 minutes after administration. The radioactivity in the stomach and small intestine, and the contents of the stomach and small intestine was measured. Radioactivity concentrations in the samples were analyzed, and samples were profiled by — HPLC analyses.

Results: Following oral administration of a 50 µg/kg dose of ^{3}H-RU-0211, plasma concentration reached maximum level at 30 minutes after administration (0.5% of the dose). In the

gastrointestinal tract, the highest radioactivity concentration was observed in the stomach. Most of the administered radioactivity was detected in the stomach contents and the stomach. In the stomach, 15-hydroxy-RU-0211 (C) and 2, 3, 4, 5-tetranor-RU-0211 (D) were also detected in addition to the parent compound. In the small intestinal contents and jejunum, C was detected as a major metabolite, and the parent compound was hardly detected. In the plasma, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 (J) was detected as a major metabolite, while C and 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 (J) were detected in small amounts. Radioactivity concentrations in different tissues after oral administration of a 50 µg/kg dose of RU-0211 to male rats are shown in the Table below.

Table 2. Radioactivity Concentrations in Tissues after Oral Administration of 50 µg/kg of ^{3}H-RU-0211 to Rats

Tissues	Radioactivity concentration (ng eq./g)*			
	5 min	10 min	15 min	30 min
Plasma	0.68	0.63	0.82	7.93
Stomach	NT	888.37	1859.60	1453.40
Duodenum	NT	NT	NT	NT
Jejunum	NT	42.22	14.80	72.50
Ileum	NT	NT	NT	NT

NT: Not Tested

*: plasma concentration was expressed as ng eq./mL

The metabolites in the plasma and gastrointestinal tract of male rats following oral administration of a 50 µg/kg dose of RU-0211 is shown in the Table below.

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Table 4.
Metabolites in Plasma and Gastrointestinal Tract After Single Oral Administration of 50 µg/kg ^{3}H-RU-0211 to Male Rats

Time (minutes)	5	10	15	30
Plasma*				
Plasma Concentration (ng eq./mL)	0.68	0.63	0.82	7.93
% of Dose	0.0**	0.0**	0.0**	0.5
Metabolites (%)				
• Parent RU-0211	NT	NT	NT	2.17
• 15-OH-RU-0211(C)	NT	NT	NT	6.96
• 2,3,4,5-tetranor-RU-0211 (D)	NT	NT	NT	9.13
• 19-carboxy[a] (J)	NT	NT	NT	38.26
Stomach Contents				
% of Dose	NT	47.9	37.5	21.0
Metabolites (%)				
• Parent RU-0211	NT	90.92	97.53	91.39
• 15-OH-RU-0211(C)	NT	0.48	0.15	0.18
• 2,3,4,5-tetranor-RU-0211 (D)	NT	#	#	0.10
• 19-carboxy[a] (J)	NT	#	#	0.76
Stomach Tissue				
% of Dose	NT	11.0	23.0	19.4
Metabolites (%)				
• Parent RU-0211	NT	11.53	30.73	65.11
• 15-OH-RU-0211(C)	NT	60.00	34.44	22.83
• 2,3,4,5-tetranor-RU-0211 (D)	NT	9.64	2.28	1.25
• 19-carboxy[a] (J)	NT	#	#	0.91
Small Intestine Contents				
% of Dose	0.2	1.4	0.6	13.8
Metabolites (%)				
• Parent RU-0211	4.24	18.74	#	1.25
• 15-OH-RU-0211(C)	55.93	59.58	69.01	32.62
• 2,3,4,5-tetranor-RU-0211 (D)	#	#	#	0.67
• 19-carboxy[a] (J)	#	2.74	#	28.14
Jejunum				
% of Dose	NT	0.4	0.1	0.6
Metabolites (%)				
• Parent RU-0211	NT	#	#	#
• 15-OH-RU-0211 (C)	NT	84.21	59.63	64.48
• 2,3,4,5-tetranor-RU-0211 (D)	NT	2.19	#	#
• 19-carboxy[a] (J)	NT	#	#	1.46

* whole weight of plasma regarded as 3.13% of the body weight
** less than 0.1
[a] 19-carboxy-15-hydroxy-2,3,4,5,20-pentanor-RU-0211
NT – Not Tested
- Not Detected

The results show that RU-0211 is rapidly metabolized in the gastrointestinal tract of male rats.

Metabolite Identification of RU-0211 after Oral Administration in Rats (Study Report #SPI/SR04-001; Cross reference #EIH120)

Methods: Plasma and urine metabolites of RU-0211 were analyzed following oral administration of ^{3}H-RU-0211 (mini capsules) to male rats at a dose of 5 mg/kg. Blood samples were collected at 1 and 2 hours after dosing from 2 animals at each time-point. 0-6 hr and 0-24 hr urine samples were collected from 3 animals at each time point. Metabolic profiles in plasma and urine were investigated by high-performance liquid chromatography (HPLC), and metabolite identification was performed by tandem mass spectrometry (MS/MS).

Results: Following oral administration of ^{3}H-RU-0211 to male rats, the mean radioactivity concentrations in plasma were 53.75 ng eq./ml at 1 hr and 111.85 ng eq./ml at 2 hrs after dosing. At 2 hours after oral dosing, metabolite peaks D, E, H, J and K were prominent peaks (constituting >5% of sample radioactivity) in plasma. These peaks represented 9.31%, 9.48%, 9.83%, 36.9% and 5.69% of the radioactivity of the sample. In the plasma, the metabolite peak C was detected in small amounts. The metabolites C, D, H, J and K are thought to correspond to metabolites M3, M4, M12, M14 and M15 detected in human plasma and/or urine, respectively. MS/MS analyses of the plasma samples revealed that 15-hydroxy-RU-0211, 2, 3, 4, 5-tetranor-RU-0211, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 PGA type, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 and 15, 20-dihydroxy-2, 3, 4, 5-tetranor-RU-0211 corresponded to peak corresponded to peaks C, D, H1 (peak H consisted of two peaks, H1 and H2), J and K, respectively. The structures of the parent compound and the metabolites are shown in the Figure below.

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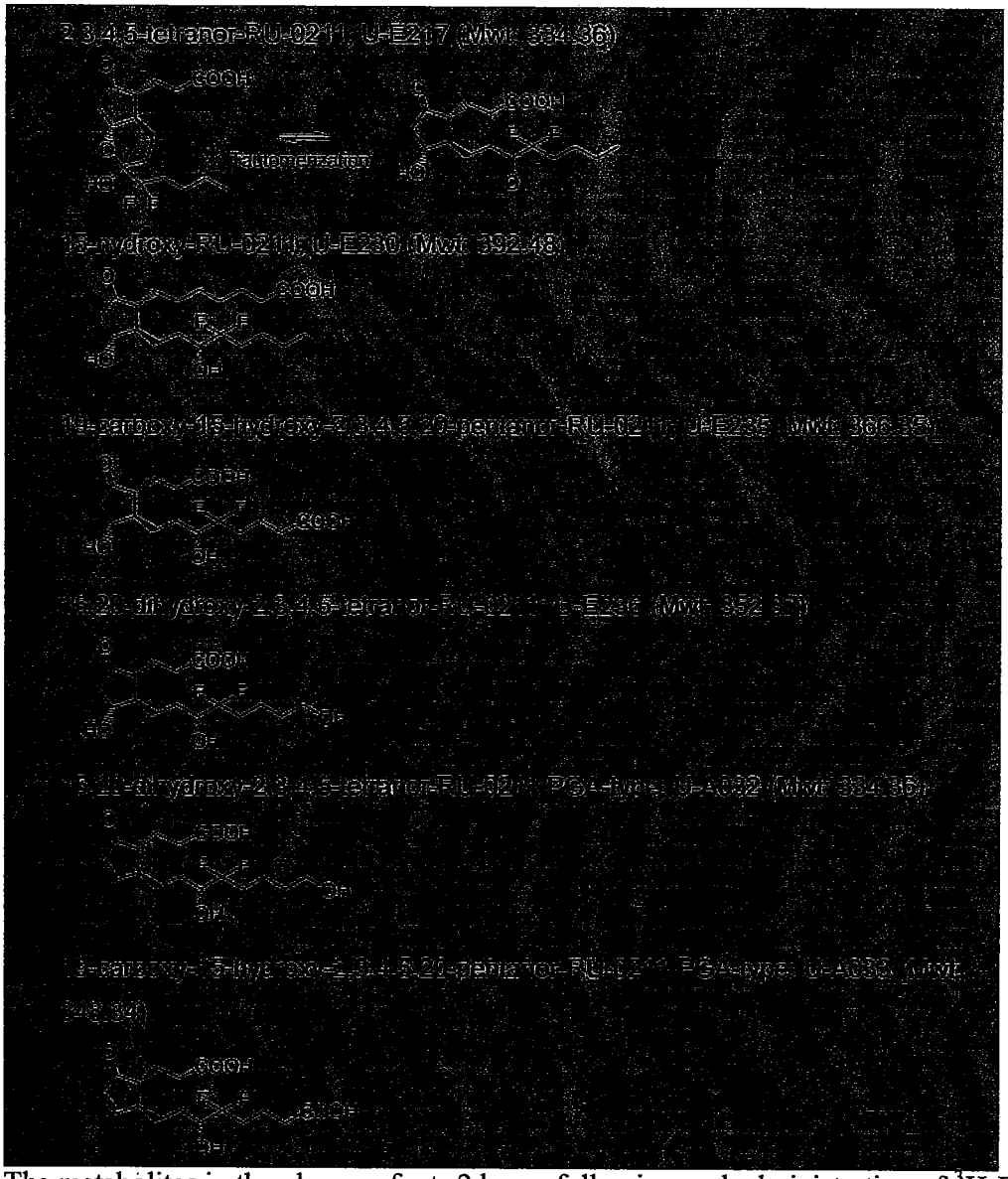

The metabolites in the plasma of rats 2 hours following oral administration of ^{3}H-RU-0211 is shown in the Table below.

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Metabolites in Plasma at 2 Hours after Oral Administration of ^{3}H-RU-0211 to Rat (Animal No. 2-1)

Metabolite	Retention Time (min)	Radioactivity [a] (cpm)	Percent Sample Radioactivity in Metabolite Peak (concentration in ng eq./mL)
RU-0211	[illegible]	[illegible]	ND [b] (ND)
B	37.00	156	4.48 (4.76)
C	[illegible]	[illegible]	ND (ND)
D	30.80	324	[illegible] (9.38)
E	24.[illegible]	[illegible]	[illegible] ([illegible])
F	19.90	54	[illegible] ([illegible])
G	[illegible]	142	[illegible] ([illegible])
H	10.50	55	[illegible] (2.32)
J	7.80	[illegible]	[illegible] ([illegible])
K	7.51	[illegible]	[illegible] ([illegible])
L	[illegible]	[illegible]	ND (ND)
[illegible]	[illegible]	[illegible]	ND (ND)
Total Sample Radioactivity		[illegible]	[illegible]

[a] The on-line radioactivity detection limit was [illegible] cpm

[b] Not detected

Radioactivity concentration in each metabolite peak was calculated from the total radioactivity concentration and the percent of the radioactivity in each metabolite peak.

The radioactivity concentrations in the urine were 52,542 ng eq./g in the 0-6 hr sample and 29,051 ng eq./g in the 6-24 hr sample. The cumulative excretion in the urine in 24 hrs was 11.53%. In urine collected from 0-6 hours after dosing, metabolite peaks H, J and K were prominent peaks (representing 16.54%, 44.1% and 16.0% of the radioactivity in the sample, respectively). MS/MS analyses of the urine samples also revealed that 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 PGA type, 15, 20-dihydroxy-2, 3, 4, 5-tetranor-RU-0211 PGA type, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 and 15, 20-dihydroxy-2, 3, 4, 5-tetranor-RU-0211 corresponded to peaks H1, H2, J and K, respectively.
The metabolites detected in the 0-6 hrs urine samples of rats administered ^{3}H-RU-0211 are shown in the Table below.

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Metabolites in Urine at 0 to 6 Hours after Oral Administration of ^{3}H-RU-0211 to Rat (Animal No. 3-3)

Metabolite	Retention Time (min)	Radioactivity[a] (cpm)	Percent Sample Radioactivity in Metabolite Peak (percent of dose)
RU-0211	45	5	ND[b] (ND)
B	36	12	ND (ND)
C	[illegible]	0	ND (ND)
[illegible]	30	5	ND (ND)
E	21.40	108	[illegible] (0.05)
[illegible]	[illegible]	55	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	254	[illegible]
[illegible]	[illegible]	4,464	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
Dose in Urine	[illegible]	[illegible]	[illegible]

a The on-line radioactivity detection limit was [illegible] cpm.

b Not detected

Radioactivity excretion in each metabolite peak was calculated from the total radioactivity excretion and the percent of the radioactivity in each metabolite peak.

The results suggest that in rats, RU-0211 is initially metabolized by ? -oxidation of the ? chain following reduction of 15-carbonyl group and ß-oxidation of the a-chain. Some metabolites are further metabolized from a PGE-type structure to a PGA-type structure by dehydration. Thus, the metabolism of RU-0211 in rats is similar to the metabolism of natural prostaglandin E_1.
The proposed metabolic pathway of RU-0211 in rats is shown in the Figure below.

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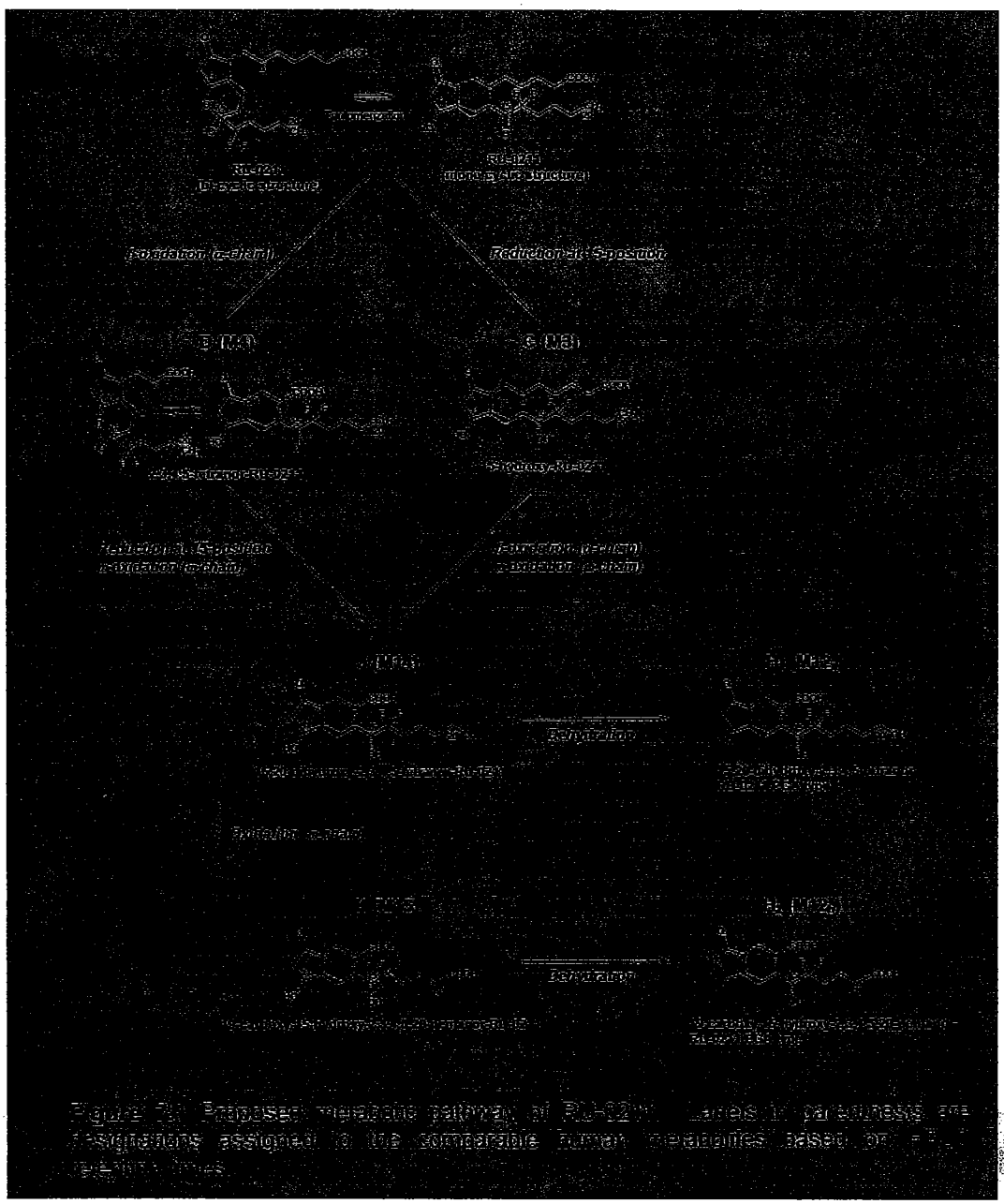

Metabolite Profiling in Rat and Dog Plasma after Oral Administration of RU-0211 (Study Report #SPI/SR05-002)

Methods: Metabolite profiles of RU-0211 were determined following oral administration of ^{3}H-RU-0211 in rats and dogs. ^{3}H-RU-0211 was administered to male rats and male beagle dogs at doses of 50 µg/kg and 5µg/kg, respectively. In rats, samples of blood were collected at 10, 15, 30, 45 min and 1, 1.5 and 2 hours following administration of the dose. In dogs, blood samples were

collected at 30 and 45 min and 1, 1.5, 2, 3, 4 and 8 hours after dosing. Radioactivity in all samples was measured by liquid scintillation counting and the metabolite profiles of RU-0211 determined by — HPLC analysis.

Results: Following oral administration of a 50 μg/kg dose of ^{3}H-RU-0211 to rats, the mean radioactivity concentration in plasma reached maximum levels at 0.25 hr after administration, and then declined. The mean C_{max} value was 10.6 ng eq./ml, and the mean AUC_{0-a} was 63.4 ng eq.hr/ml. The plasma pharmacokinetic parameters of the radioactivity in rats following oral administration of ^{3}H-RU-0211 is shown in the Table below.

Pharmacokinetic Parameters in Plasma after Oral Administration of ^{3}H-RU-0211 to Rats

Pharmacokinetic parameters		
C_{max}	(ng eq./mL)	10.60
T_{max}	(hr)	0.25
$T_{1/2}$*	(hr)	2.55
$AUC_{0\text{-last t}}$	(ng eq.·hr/mL)	10.2
$AUC_{0-\infty}$	(ng eq.·hr/mL)	28.0
AUC_{0-24}**	(ng eq.·hr/mL)	63.4

Data are calculated from the mean plasma concentration from three animals sampled at each time point.

NC: Not calculated

* Calculated from 0.75 hour to 2 hours

** Plasma concentration at 24 hours was regarded as 0 ng eq./mL.

At all time points of determination in rats, unchanged RU-0211 detected (retention time, about 47 minutes). Peaks D, E, F, H and J (retention times of about 33, 23, 19, 13 and 8 minutes, respectively) were detected as predominant peaks (>5% of the total radioactivity eluted) in rat plasma. At all time points, peak C (retention time, about 35 min) was detected with 1.80% to 3.51% of the total radioactivity eluted. The metabolite profiles in the rat plasma at 1.0, 1.5 and 2.0 hrs after administration of ^{3}H-RU-0211 are shown in the Table below.

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Metabolites in Plasma after Oral Administration of ^{3}H-RU-0211 to Rats

Metabolite	Retention Time (min)	Mean Percent Sample Radioactivity in Metabolite Peak (Concentration in ng eq./mL)		
		1 hr	1.5 hrs	2 hrs
Sample Radioactivity		(4.44 ± 2.30)	(3.43 ± 1.18)	(4.84 ± 0.35)
RU-0211	47	1.78 ± 1.57 (0.09 ± 0.09)	1.78 ± 0.52 (0.06 ± 0.03)	1.10 ± 0.38 (0.05 ± 0.02)
A	43	0.40 ± 0.21 (0.02 ± 0.02)	0.35 ± 0.03 (0.01 ± 0.00)	0.40 ± 0.13 (0.02 ± 0.00)
B	39	0.37 ± 0.12 (0.02 ± 0.01)	0.46 ± 0.09 (0.02 ± 0.00)	0.39 ± 0.07 (0.02 ± 0.00)
C	35	2.06 ± 0.75 (0.09 ± 0.06)	2.60 ± 1.03 (0.08 ± 0.02)	1.80 ± 0.39 (0.09 ± 0.02)
D	33	7.49 ± 3.06 (0.38 ± 0.30)	9.10 ± 2.75 (0.33 ± 0.19)	6.15 ± 1.87 (0.29 ± 0.07)
N	31	1.70 ± 0.45 (0.08 ± 0.06)	1.97 ± 0.72 (0.06 ± 0.01)	2.37 ± 0.31 (0.11 ± 0.01)
E	23	7.26 ± 2.18 (0.34 ± 0.25)	8.10 ± 0.52 (0.28 ± 0.09)	5.43 ± 0.94 (0.26 ± 0.03)
F	19	24.14 ± 6.52 (1.11 ± 0.78)	26.26 ± 0.89 (0.90 ± 0.29)	23.53 ± 0.78 (1.14 ± 0.12)
G	17	1.84 ± 0.59 (0.09 ± 0.07)	1.77 ± 0.51 (0.06 ± 0.04)	2.09 ± 0.20 (0.10 ± 0.02)
H	13	8.21 ± 1.25 (0.37 ± 0.20)	7.65 ± 1.99 (0.28 ± 0.15)	11.18 ± 1.27 (0.54 ± 0.09)
I	11	2.38 ± 0.19 (0.10 ± 0.05)	3.32 ± 0.25 (0.12 ± 0.04)	4.55 ± 1.34 (0.22 ± 0.08)
J	8	32.10 ± 9.11 (1.33 ± 0.61)	26.96 ± 2.70 (0.91 ± 0.29)	27.22 ± 2.14 (1.32 ± 0.15)
K	7	5.33 ± 2.08 (0.20 ± 0.05)	4.46 ± 0.73 (0.15 ± 0.03)	7.87 ± 0.75 (0.38 ± 0.05)
L	5	0.97 ± 0.38 (0.04 ± 0.02)	1.13 ± 0.24 (0.04 ± 0.01)	1.06 ± 0.07 (0.05 ± 0.01)
M	3	0.37 ± 0.10 (0.02 ± 0.01)	0.36 ± 0.09 (0.01 ± 0.01)	0.33 ± 0.01 (0.02 ± 0.00)
Other peaks		3.22 ± 0.76 (0.15 ± 0.10)	3.39 ± 0.91 (0.12 ± 0.07)	4.29 ± 0.48 (0.21 ± 0.03)

Quantification limit for plasma concentration was —

Radioactivity concentration in each metabolite peak was calculated from the total radioactivity concentration and the percent sample radioactivity in each metabolite peak.

In dogs, unchanged RU-0211 was detected at 0.5, 1, 1.5 and 2 hours after administration. The parent compound was not detected at any other time points. The mean radioactivity concentration reached maximum levels at 0.5 hr following administration of a 5 µg/kg dose dose. The mean Cmax was 6.31 ng eq./ml and the AUC_{0-a} was 14.5 ng eq.hr/ml. The mean pharmacokinetic parameters of the total radioactivity following oral administration ^{3}H-RU-0211 in dogs is shown in the Table below.

Mean Pharmacokinetic Parameters in Plasma after Oral Administration of ^{3}H-RU-0211 to Dogs

Pharmacokinetic parameters		
C_{max}	(ng eq./mL)	6.31 ± 0.49
T_{max}	(hr)	0.58 ± 0.14
$T_{1/2\,\alpha}$	(hr)	1.12 ± 0.02
$T_{1/2\,\beta}$	(hr)	2.48 ± 0.13
$AUC_{0\text{-last t}}$	(ng eq.·hr/mL)	12.5 ± 1.9
$AUC_{0\text{-}\infty}$	(ng eq.·hr/mL)	13.4 ± 1.9
$AUC_{0\text{-}24}$*	(ng eq.·hr/mL)	14.5 ± 2.2

Data are expressed as the means ± SD from parameters calculated individually from three animals.

* Plasma concentration at 24 hours was regarded as 0 ng eq./mL.

Peaks N, E, F, G and J (retention times of about 31, 23, 19, 17 and 8 minutes, respectively) were detected as predominant peaks at 0.5, 0.75 and 1.0 hr after administration. Similarly, peaks E, F, G and J at 1.5 and 2.0 hrs, peaks E, F, G, H, J and K at 3.0 hrs, peaks N, E, F, G, H, J and K at 4.0 hrs, peaks A, E, F, G, H, J and K at 8.0 hours after administration were detected, respectively. The percentage of RU-0211 radioactivity was 3% of the total radioactivity at the first collection time point, indicating that RU-0211 is rapidly metabolized in both species. Peaks C, D, H, J and K, were previously identified as 15-hydroxy-RU-0211, 2, 3, 4, 5-tetranor-RU-0211, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 PGA type, 19-carboxy-15-hydroxy-2, 3, 4, 5, 20-pentanor-RU-0211 and 15, 20-dihydroxy-2, 3, 4, 5-tetranor-RU-0211, respectively. The metabolite profiles of RU-0211 at 2.0, 3.0, 4.0 and 5.0 hrs following oral administration of ^{3}H-RU-0211 are shown in the Table below.

Metabolites in Plasma after Oral Administration of ^{3}H-RU-0211 to Dogs

Metabolite	Retention Time (min)	Mean Percent Sample Radioactivity in Metabolite Peak (Concentration in ng eq./mL)			
		2 hrs	3 hrs	4 hrs	8 hrs
Sample Radioactivity		(3.73 ± 0.39)	(1.08 ± 0.33)	(0.65 ± 0.18)	(0.24 ± 0.06)
RU-0211	47	0.01 ± 0.02 (0.00 ± 0.00)	ND (ND)	ND (ND)	ND (ND)
A	43	1.10 ± 0.06 (0.03 ± 0.01)	1.50 ± 0.12 (0.02 ± 0.00)	2.00 ± 0.08 (0.01 ± 0.00)	6.43 ± 0.98 (0.02 ± 0.00)
B	39	0.29 ± 0.03 (0.01 ± 0.00)	0.54 ± 0.12 (0.01 ± 0.00)	0.97 ± 0.22 (0.01 ± 0.00)	3.27 ± 0.08 (0.01 ± 0.00)
C	35	0.34 ± 0.16 (0.01 ± 0.00)	0.30 ± 0.12 (0.00 ± 0.00)	0.40 ± 0.15 (0.00 ± 0.00)	1.00 ± 0.42 (0.00 ± 0.00)
D	33	0.86 ± 0.16 (0.02 ± 0.01)	0.53 ± 0.12 (0.01 ± 0.00)	0.59 ± 0.28 (0.00 ± 0.00)	0.71 ± 0.73 (0.00 ± 0.00)
N	31	4.67 ± 1.10 (0.13 ± 0.05)	4.92 ± 1.17 (0.05 ± 0.02)	5.23 ± 0.97 (0.03 ± 0.01)	4.67 ± 0.23 (0.01 ± 0.00)
E	23	22.31 ± 3.25 (0.62 ± 0.18)	17.08 ± 2.43 (0.18 ± 0.06)	15.45 ± 1.95 (0.10 ± 0.02)	13.93 ± 2.17 (0.03 ± 0.01)
F	19	23.14 ± 2.74 (0.63 ± 0.10)	21.28 ± 1.94 (0.23 ± 0.06)	18.66 ± 2.14 (0.12 ± 0.03)	16.26 ± 1.35 (0.04 ± 0.01)
G	17	10.87 ± 2.93 (0.29 ± 0.05)	9.12 ± 2.37 (0.10 ± 0.05)	8.50 ± 1.76 (0.06 ± 0.02)	11.18 ± 1.65 (0.03 ± 0.01)
H	13	3.93 ± 0.44 (0.11 ± 0.03)	5.20 ± 0.77 (0.06 ± 0.01)	6.23 ± 1.24 (0.04 ± 0.01)	7.53 ± 1.51 (0.02 ± 0.01)
I	11	1.96 ± 0.30 (0.05 ± 0.02)	2.75 ± 0.65 (0.03 ± 0.01)	3.76 ± 1.06 (0.03 ± 0.01)	2.88 ± 1.47 (0.01 ± 0.01)
J	8	23.91 ± 0.71 (0.65 ± 0.08)	25.88 ± 1.55 (0.28 ± 0.10)	23.01 ± 1.95 (0.15 ± 0.05)	11.55 ± 2.11 (0.03 ± 0.01)
K	7	4.07 ± 1.16 (0.11 ± 0.02)	6.95 ± 1.60 (0.07 ± 0.02)	9.62 ± 0.73 (0.06 ± 0.02)	6.47 ± 0.91 (0.02 ± 0.00)
L	5	0.18 ± 0.02 (0.00 ± 0.00)	0.38 ± 0.04 (0.00 ± 0.00)	0.63 ± 0.05 (0.00 ± 0.00)	0.85 ± 0.22 (0.00 ± 0.00)
M	3	0.10 ± 0.01 (0.00 ± 0.00)	0.17 ± 0.02 (0.00 ± 0.00)	0.31 ± 0.07 (0.00 ± 0.00)	0.13 ± 0.22 (0.00 ± 0.00)
Other peaks		2.01 ± 0.21 (0.05 ± 0.00)	2.72 ± 0.48 (0.03 ± 0.01)	3.41 ± 0.96 (0.02 ± 0.01)	8.65 ± 0.47 (0.02 ± 0.01)

Quantification limit for plasma concentration was — ng eq./mL.

ND: Not detected

Radioactivity concentration in each metabolite peak was calculated from the total radioactivity concentration and the percent sample radioactivity in each metabolite peak.

These results support the metabolic pathway revealed previously; reduction of 15 carbonyl group, ß-oxidation of a chain, ? -oxidation of ? chain, and further oxidation and transformation from PGE type to PGA type.

Evaluation of Cytochrome P450 Involved in the Biotransformation of RU-0211 to 15-Hydroxy-RU-0211 (Study Report # SPI/SR05-006).

Methods: The potential role of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP4A11 in the biotransformation of RU-0211 to 15-hudroxy-RU-0211 was investigated in pooled human microsomes (with CYP specific inhibitors) and in Supersomes (cDNA expressed microsomes) expressing the CYP isoforms. The following CYP inhibitors were used in the study:

P450 Isoform	Specific Inhibitor	Final Concentration (μM)
CYP1A2	Furafylline	50
CYP2A6	Pilocarpine	100
CYP2B6	Thio-TEPA	50
CYP2C8	Quercetin	3
CYP2C9	Sulfaphenazole	10
CYP2C19	(S)-Mephenytoin	360
CYP2D6	Quinidine	1
CYP2E1	4-Methylpyrazole	300
CYP3A4	Ketoconazole	1
CYP4A11	Lauric Acid	100
CYP4A11	17-Octadecynoic Acid	1, 10, and 25
Non-Specific	1-Aminobenzotriazole	100

Based upon preliminary incubations, a protein concentration 0.1 mg/ml and an incubation time of 10 minutes were used. The concentration of 15-hydroxy-RU-0211 in the incubate was determined by LC/MS/MS analysis.

Results: Inhibition experiments using pooled human microsomes and CYP specific chemical inhibitors did not show any significant inhibition of formation of 15-hydroxy-RU-0211 by any CYP-specific inhibitor, with the exception of lauric acid (a CYP4A11 inhibitor). In addition, furafylline (CYP1A2 inhibitor) and quercetin (CYP2C8 inhibitor) caused slight inhibitions of formation of 15-hydroxy-RU-0211. It was thought that the inhibiton caused by lauric acid could be formation of micelles and thus changing the test article solubility/concentration. Therefore, a repeat incubation was performed using another known inhibitor of CYP4A11 (17-octadecynoic acid, ODYA). ODYA did not show a significant inhibition of in 15-hydroxy-RU-0211 formation, indicating CYP4A11 is not likely be involved in the metabolism of RU-0211 to 15-hydroxy-RU-0211. The effects of different CYP inhibitors on the formation of 15-hydroxy-RU-0211 are shown in the Table below.

Table 2. Inhibition of 15-Hydroxy-RU-0211 Formation by Specific Inhibitors at 100 and 1000 ng/mL RU-0211 in Human Liver Microsomes

Treatment	100 ng/mL RU-0211			1000 ng/mL RU-0211		
	Concentration (ng/mL)	Mean (ng/mL)	% Inhibition	Concentration (ng/mL)	Mean (ng/mL)	% Inhibition
Control	45.2			177		
	39.2			202		
	50.4			203		
	41.2			224		
	40.3			210		
	43.2	43.3		240	209	
Furafylline	41.7			189		
	40.6			177		
	42.1	41.5	4.2	183	183	12.3
Pilocarpine	40.6			171		
	51.1			193		
	35.9	42.5	1.7	221	195	6.8
Thio-TEPA	54.0			188		
	49.1			222		
	37.1	46.7	-8.0	240	217	-3.5
Quercetin	34.6			169		
	45.8			180		
	43.7	41.4	4.3	169	173	17.4
Sulfaphenazole	42.5			195		
	57.7			217		
	42.5	47.6	-10.1	198	204	2.7
(*S*)-Mephenytoin	35.3			198		
	39.3			211		
	37.0	37.2	14.0	189	199	4.8
Quinidine	48.1			227		
	41.7			207		
	46.7	45.5	-5.2	199	211	-0.8
4-Methlypyrazole	43.5			215		
	49.7			247		
	44.5	45.9	-6.1	254	238	-14.0
Ketoconazole	38.7			217		
	33.3			207		
	31.9	34.6	19.9	224	216	-3.3
Lauric Acid	16.1			98.3		
	16.2			87.5		
	13.9	15.4	64.4	84.0	90.0	57.0
ABT	34.9			214		
	34.6			248		
	34.0			248		
	30.3			238		
	28.5			232		
	27.1	31.5	27.1	196	229	-9.7

Note: Calculations performed with machine precision of Microsoft Excel 97.

Incubation of 100 ng/ml of RU-0211 with various expressed enzymes did not show any substantial amount of 15-hydroxy-RU-0211 formation by any of the 10 CYP enzymes used in the study. However, incubation with 1000 ng/ml of RU-0211 showed small amounts of 15-hydroxy-RU-0211 formation by CYP2A6 and CYP2B6. The amounts of 15-hydroxy-RU-0211 formation by CYP2A6 and CYP2B6 at 1000 mg/ml were approximately 2-fold higher than the control microsome containing no expressed enzymes. Thus, the results suggest that CYP2A6 and CYP2B6 may have minor roles in the conversion of RU-0211 to 15-hydroxy-RU-0211. The

metabolism of RU-0211 (1000 ng/ml) to 15-hydroxy-RU-0211 by the Supersomes is shown in the Tables below.

Metabolism of RU-0211 (at 1000 ng/mL) to 15-Hydroxy-RU-0211 Using 15 pmol/mL Supersome™

Isoform	Sample Name		Conc. (ng/mL)	Average (ng/mL)	Product Formed (ng/mL)
Control	Inst Ctrl +NADPH		24.8		
			26.3		
			27.2	26.1	26.1
	Inst Ctrl -NADPH		<1.00		
			<1.00		
		+	1.81	<1.00	
1A2	1A2 +NADPH		15.1		
			15.5		
			13.5	14.7	14.7
	1A2 -NADPH		<1.00		
			<1.00		
			<1.00	<1.00	
2D6	2D6 +NADPH		23.4		
			25.0		
			19.7	22.7	22.7
	2D6 -NADPH		<1.00		
			<1.00		
			<1.00	<1.00	
2E1	2E1 +NADPH		4.73		
			4.85		
			4.20	4.60	4.60
	2E1 -NADPH		<1.00		
			<1.00		
			<1.00	<1.00	
3A4	3A4 +NADPH		9.40		
		+	24.5		
			9.59	9.50	9.50
	3A4 -NADPH		<1.00		
			<1.00		
			<1.00	<1.00	
4A11	4A11 +NADPH		22.7		
			20.6		
			23.5	22.3	22.3
	4A11 -NADPH		<1.00		
			<1.00		
			<1.00	<1.00	

+ Not included in calculations, outlier.
Note: Calculations performed with machine precision of Microsoft Excel 97.

Metabolism of RU-0211 (at 1000 ng/mL) to 15-Hydroxy-RU-0211 Using 15 pmol/mL Supersome™

Isoform	Sample Name	Conc. (ng/mL)	Average (ng/mL)	Product Formed (ng/mL)
Control	Inst Ctrl +NADPH	29.0		
		27.4		
		37.1	31.2	31.2
	Inst Ctrl -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	
2C19	2C19 +NADPH	9.20		
		7.73		
		9.38	8.77	8.77
	2C19 -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	
2A6	2A6 +NADPH	51.2		
		77.5		
		49.7	59.5	59.5
	2A6 -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	
2B6	2B6 +NADPH	39.1		
		68.1		
		55.5	54.2	54.2
	2B6 -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	
2C8	2C8 +NADPH	3.87		
		4.61		
		4.04	4.17	4.17
	2C8 -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	
2C9	2C9 +NADPH	7.00		
		7.97		
		8.10	7.69	7.69
	2C9 -NADPH	<1.00		
		<1.00		
		<1.00	<1.00	

Note: Calculations performed with machine precision of Microsoft Excel 97.

In Vitro Human Cytochrome P450 Inhibitory Drug-Drug Interaction Study with SPI-0211 (RU-0211) (Study Report #SPI/SR04-009)

Methods: The potential of SPI-0211 to inhibit eight specific isoforms of cytochrome P450 was investigated in pooled human microsomes. The IC50 values for CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 were determined using the relevant subsrates (phenacetin, coumarin, bupropion, tolbutamide, (S)-mephenytoin, dextromethorphan, chlorzoxazone, midazolam and testosterone, respectively) at single concentrations approximating their respective apparent K_m values. The concentrations of RU-0211 used were 0.1, 1, 10, 100, 1000 and 10000 pg/ml. The metabolite formation for each enzyme activity was monitored by a validated LC-MS/MS method, and the specificity for these experimental conditions for an appropriate metabolite formation was also evaluated in the presence of specific inhibitors. Samples for mechanism-based inhibition screening were preincubated for 15 minutes at 37^0C with RU-0211 (10 or 100 pg/ml) in the presence or absence of NAPDH. The percent remaining activity of microsomes preincubated for 15 minutes at 370C with RU-0211 and NADPH were compared with microsomes preincubated with RU-0211 without NADPH.

Results: Incubation of RU-0211 with human hepatic microsomes at concentrations up to 10000 pg/ml did not cause a significant inhibition of any of the eight human cytochrome P450 isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) examined. The effects of RU-0211 on the activity of different CYP enzymes are shown in the Table below.

			Concentration of (pg/mL) RU-0211						
Isoform	**Substrate**	**Reaction**	**0.1**	**1**	**10**	**100**	**1000**	**10000**	**IC_{50}**
CYP1A2	Phenacetin	*O*-Deethylation	101	101	104	100	97.3	98	NI
CYP2A6	Coumarin	7-Hydroxylation	94	91.8	93.1	85.6	89.5	86.7	NI
CYP2B6	Bupropion	Hydroxylation	99.1	100	97.7	97.4	98.9	97.3	NI
CYP2C9	Tolbutamide	Hydroxylation	103	101	99	104	98.2	96.6	NI
CYP2C19	(S)-Mephenytoin	4'-Hydroxylation	95.9	92.8	93.4	89.1	91.5	85.8	NI
CYP2D6	Dextromethorphan	*O*-Demethylation	113	114	107	104	119	106	NI
CYP2E1	Chlorzoxazone	6-Hydroxylation	87.8	109	112	108	109	108	NI
CYP3A4	Midazolam	1'-Hydroxymidazolam	167	161	169	173	160	171	NI
CYP3A4	Testosterone	6β-Hydroxylation	94.8	100	104	101	106	101	NI

NI: No inhibition exceeding 50% was observed within the 0.1-10000 pg/mL concentration range of RU-0211.

Thus, RU-0211 does not appear to have a potential for drug-drug interactions due to mechanism-based inhibition of CYP enzymes.

In Vitro Assessment of the Enzyme Induction Potential of RU-0211 in Primary Cultures of Human Hepatocytes (Study Report # SPI/SR04-018)

Methods: The potential of RU-0211 to induce cytochromes P450 (CYP1A2, CYP2B6, CYP2C9 and CYP3A4) was examined in primary cultures of human hepatocytes. Evaluation of the degree of cytochrome P450 induction was performed by measuring enzyme activities as well as protein expression in different treatment groups. Freshly isolated human hepatocytes from three donors were cultured on collagen-coated plates and incubated with positive controls, vehicle controls, or

RU-0211 for 72 hours. RU-0211 was evaluated at 5, 30, 150 and 1000 pg/ml concentrations. Activity levels of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 were measured using phenacetin, bupropion, tolbutamide and testosterone, respectively, as substrates using validated LC-MS/MS methods. Expression of proteins was measured Western Blot analysis.

Results: Significant increases in the respective enzyme activities and protein expressions were observed with the positive controls (3-methylchloranthrene, phenobarbital, rifampin and rifampin for CYPs 1A2, 2B6, 2C9 and 3A4, respectively). Hepatocytes treated with RU-0211 at concentrations up to 1000 pg/ml did not show a significant increase in CYP1A2, CYP2B6, CYP2C9 or CYP3A4 activity. Western blot analysis of homogenates from hepatocytes treated with RU-0211 did not show an increase in the expression of the enzyme proteins at any concentration. The effects of the positive controls and different concentrations of RU-0211 on CYP induction are shown in the Table below.

Table S1: Summary of Fold Change of Cytochrome P450 Activity in Human Liver Hepatocytes Treated with Control Inducers or RU-0211 as Compared to the Controls

Isoform (positive control)	Donor	Fold Change				
		Positive Control	RU-0211			
			5 pg/mL	30 pg/mL	150 pg/mL	1000 pg/mL
1A2 (3-Methylcholanthrene)	1	67.0	1.3	1.4	1.2	1.4
	2	10.7	0.9	0.8	0.8	0.7
	3	15.1	0.9	1.2	1.0	1.2
2B6 (Phenobarbital)	1	6.4	0.8	0.9	1.1	0.9
	2	24.3	1.2	0.9	1.4	1.5
	3	9.9	0.8	1.1	1.0	0.7
2C9 (Rifampin)	1	1.9	0.9	1.2	1.0	1.1
	2	2.3	0.9	0.9	1.0	1.0
	3	2.7	1.0	0.9	1.0	1.1
3A4 (Rifampin)	1	5.0	0.7	1.5	1.3	1.4
	2	11.2	0.9	1.5	1.1	1.5
	3	4.4	1.2	1.2	1.3	1.4

Thus, RU-0211 had no potential for induction of CYP1A2, CYP2B6, CYP2C9 or CYP3A4 activity in human hepatocytes at concentrations up to 1000 pg/ml.

Investigation of Involvement of Microsomal Carbonyl Reductase in the Biotransformation of RU-0211 to 15-hydroxy-RU-0211 (Study Report #SPI/SR05-015)

Methods: To evaluate possible involvement of microsomal carbonyl reductases in the conversion of RU-0211 to 15-hydroxy-RU-0211, RU-0211 (100 and 1000 ng/ml) was incubated with human liver microsomes and NADPH in the presence of three different inhibitors of microsomal carbonyl reductase (quercitrin, indomethacin and glycyrrhetinic).

Results: In pooled human liver microsomes, carbonyl reductase inhibitors, quercitrin and glycyrrhetinic caused >50% inhibition of 15-hydroxy-RU-0211 formation at both 100 and 1000

ng/ml concentrations of RU-0211. Indomethacin also caused an inhibition of formation of 15-hydroxy-RU-0211 from RU-0211; however, the inhibition was <50% at the concentration used (125 μM). The inhibition of the formation of 15-hydroxy-RU-0211 from RU-0211 by the carbonyl reeducates inhibitors suggests the involvement of microsomal carbonyl reductase or similar enzymes in the formation of this metabolite. The effect of carbonyl reductase inhibitors on the biotransformation of RU-0211 (100 and 1000 ng/ml) to 15-hydroxy-RU-0211 is shown in the Table below.

	100 ng/mL RU-0211			1000 ng/mL RU-0211		
Treatment	Concentration 15-Hydroxy-RU-0211 (ng/mL)	Average Concentration (ng/mL)	Average % Remaining Activity	Concentration 15-Hydroxy-RU-0211 (ng/mL)	Average Concentration (ng/mL)	Average % Remaining Activity
Control	31.3			241		
	31.1			202		
	28.7			179		
	35.7			210		
	30.1			201		
	30.1	31.2	100	190	204	100
Quercetin (100 μM)	14.0			71.2		
	14.1			69.8		
	14.0			71.1		
	14.4			73.5		
	13.2			68.6		
	14.0	14.0	44.7	70.1	70.7	34.7
Indomethacin (125 μM)	22.7			147		
	23.4			144		
	23.0			144		
	23.9			136		
	22.1			132		
	22.5	22.9	73.5	136	140	68.7
Glycyrrhetinic (8 μM)	10.2			69.7		
	9.67			68.2		
	11.2			68.6		
	10.3			67.1		
	10.7			66.8		
	10.2	10.4	33.3	68.3	68.1	33.4

2.6.4.6 Excretion

Following administration of ^{3}H-RU-0211 to rats, mice and dogs, the radioactivity is excreted by both the renal and fecal routes. In rats, following intravenous administration of a 50 μg/kg doses, 51.6% of the radioactivity was excreted in the urine and 32% in the feces within 48 hours after administration. Following oral administration of 25, 50 and 100 μg/kg doses to rats, 25.8%, 26.2% and 27.0% of the radioactivity were excreted in the urine, and 47.3%, 52.4% and 48.7% of the radioactivity were excreted in the feces, respectively. In mice, following oral administration of 25 and 50 μg/kg doses of ^{3}H-RU-0211, 57.6% and 51.5% of the radioactivity were excreted in the urine, and 17.9% and 16.8% of the radioactivity were excreted in the feces, respectively, in 48 hours. In the dog, within 72 hours after oral administration of a 5 μg/kg dose of ^{3}H-RU-0211, 70.3% of the administered radioactivity was excreted in the urine and 14.4% of the radioactivity was excreted in the feces. In the monkey, the major route of elimination of the ^{3}H-RU-0211-related radioactivity was via the renal pathway (72.5% in 48 hours). Thus, it appears that the major route of excretion of ^{3}H-RU-0211 is via the renal route in all species, except in rats following oral administration. This may be due to incomplete absorption of the parent compound or the metabolites from the gastrointestinal tract of rats.

2.6.4.7 Pharmacokinetic drug interactions

RU-0211was not metabolized by the hepatic microsomal CYP enzymes, and it was found not to inhibit or activate any CYP isozymes. Thus, RU-0211 is not expected to have any potential for pharmacokinetic drug interactions via these pathways.

2.6.4.8 Other Pharmacokinetic Studies

HPLC Profiles of Metabolite M3 in Human Plasma and Peak C in Rat Plasma after Oral Administration of ^{3}H-RU-0211 (EIH103-A1)

Methods: Following oral administration of RU-0211 to rats and humans, 15-hydroxy-RU-0211 has been identified as an early metabolite in the plasma. In humans, this metabolite was designated as M3 and in rats it was designated as peak C. Although, 15-hydroxy-RU-0211 exists in two possible epimeric forms, it elutes as a single peak in the — HPLC. In the present study, the presence of the individual 15-hydroxy-RU-0211 epimers was evaluated in human and rat plasma samples following oral administration of 3H-RU-0211 (3.96 mCi/mg, Lot #2; radiochemical purity, —) using — HPLC. In humans, plasma samples were collected at 1.5 hours after administration of a dose of 72 µg/person. In rats, plasma samples were collected at 5.0 minutes after administration of a 50 µg/kg dose.

Results: After oral administration of RU-0211 to humans, the metabolite M3 (identified as 15-hydroxy-RU-0211) was identified. In rats, the metabolite, designates as peak C was found to have similar retention time to that of human M3, and was also thought to be 15-hydroxy-RU-0211. Further profiling of 15-hydroxy-RU-0211 under — HPLC — two individual epimeric peaks were identified. These peaks, defined as the high polar epimer (U-E232) and the low polar epimer (U-E231) had retention times of 23.5 and 20.9 minutes, respectively.

Based on the retention times of the metabolite peaks, it was concluded that human M3 and rat peak C were the same metabolite (15-hydroxy-RU-0211) and that both epimeric forms of the metabolite were detected in the two species.

2.6.4.9 Discussion and Conclusions

RU-0211 was rapidly absorbed in rats, mice and dogs following oral administration. Following oral administration of single doses to rats, the maximum plasma concentrations were reached in 0.5 to 0.75 hr, and the terminal elimination half-lives ($t_{1/2}$) ranged from 2 to 6 hours. In mice, the T_{max} was 10 to 15 minutes, and the $t_{1/2}$ was about 3 hours. In contrast to rats and mice, in dogs the T_{max} for the total radioactivity was 3 hours following oral administration, and the $t_{1/2}$ was longer than in rats. In rats, there were no signs of accumulation of the drug in any specific tissue. The main metabolite of RU-0211, 15-hydroxy-RU-0211 was pharmacologically active in an in vitro assay. It also caused a dose-dependent intestinal fluid excretion in rats following oral

administration, with potency similar to that of the parent compound. No other metabolite showed pharmacological activities. Microsomal CYP enzymes had no major role in the metabolism of RU-0211. Thus RU-0211 has minimal potential for drug interactions by inhibiting or activating hepatic microsomal enzymes. The sponsor did not examine whether the drug or its active metabolite is excreted in the milk of lactating animals. Thus, care should be taken in the use of the drug by lactating mothers, because there is a potential for causing diarrhea in breast-feeding children by transfer of the drug and/or metabolite via milk.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.5 TOXICOLOGY

Study title: Single Dose Oral Toxicity Study of RU-0211 in Rats and Dogs.

Sponsor's ID # 403415 and 600116

Conducting laboratory (and location if not Sponsor):

Dates of study initiation & completion: February 09, 1996 & March 21, 1997,
and March 18, 1996 & March 21, 1997

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Methods:

The doses used in the rat study were based on a preliminary dose range finding study in which a single oral dose of 125 mg/kg caused death of 100% (2/2) of the animals; so, the high dose was selected as 120 mg/kg and the mid- and the low doses were ½ and 1/4th of the high dose respectively.

The doses used in this single dose toxicity study in dogs were based on a preliminary study with RU-0211. A single oral dose of 20 mg/kg caused diarrhea and vomiting within 2 hours of administration. So, the high dose was selected as 40 mg/kg and the low dose was ½ the high dose.

Dosing information for rats:

Species	– CD Sprague-Dawley rats
#/sex/group or time point	5/sex/group
Age	4 weeks
Weight	Males 73-86 g; Females 64-75 g
Dosage groups in administered units	0, 30, 60 and 120 mg/kg
Route, form, volume and infusion rate (if i.v.)	RU-0211 was dissolved in PEG-400 and administered by oral gavage (5 ml/kg). The control group received PEG-400 only.

Dosing information for dogs:

Species	Beagle dogs
#/sex/group or time point	2/sex/group
Age	6.0-6.5 months
Weight	Males 8.9-9.4 kg; Females 8.2-9.3 kg
Dosage groups in administered units	0, 20 and 40 mg/kg
Route, form, volume and infusion rate (if i.v.)	RU-0211 was mixed with — — , packed in gelatin capsules and administered orally. The control animals received capsules containing — only.

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot # 4 & Lot # 5.

Formulation/vehicle: For the rat study, RU-0211 was dissolved in PEG-400 at concentrations of 6, 12 and 24 mg/ml and for the dog study, gelatin capsules containing RU-0211 plus — were used.

Times at which Observations were made:

Clinical signs- 0.5, 2, 4 and 6 hrs after dosing on the day of dosing and once a day thereafter.

Body weights- days 1, 3, 7, 10 and 14 of dosing.

Results:

Clinical signs:

Two females in the 30 mg/kg group, 1 male and 2 females in the 60 mg/kg group and all animals in the 120 mg/kg died at different times after dosing with RU-0211. The mortality data for the male and female rats receiving different doses of RU-0211 are summarized in the table below.

Mortalities in male and female rats receiving RU-0211

	Group	No. of animals	Number of deaths								Total no. of deaths	Minimum Lethal dose (MLD)
			Days after administration									
			1	2	3	4	6	8	10	14		
Male	**Control**	5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
	30 mg/kg	5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
	60 mg/kg	5	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**60 mg/kg**
	120 mg/kg	5	**2**	**0**	**3**	–	–	–	–	–	5	
Female	**Control**	5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
	30 mg/kg	5	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	
	60 mg/kg	5	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**30 mg/kg**
	120 mg/kg	5	**5**	–	–	–	–	–	–		5	

Abnormal clinical signs in the male and female rats included decreased locomotor activity (all doses), lacrimation, bradypnea and prone position 60 and 120 mg/kg doses). The body weights of the male (19%) and female (18%) rats receiving the 30 mg/kg dose were lower than controls on Day 1 of dosing; males receiving the 60 mg/kg dose had lower body weights throughout the experimental period (13.3-28%) while the females had lower body weights on Days 1 and 3 (~21%).

In dogs, there were no deaths of animals in any group. Abnormal clinical signs included decreased locomotor activity, loose stool/diarrhea, vomiting, lacrimation, salivation (except males in the 20 mg/kg group) and pale buccal mucous membrane were observed in both males and females receiving RU-0211.

Key Study Observations: In the single-dose oral toxicity study with RU-0211 in rats, the minimal lethal dose (MLD) was 30 mg/kg for the females and 60 mg/kg for the males. The clinical signs included decreased locomotor activity, clear lacrimation, loose stool and bradypnea.

In the single dose toxicity study in dogs, there were no deaths in any groups. The clinical signs included decreased locomotor activity, vomiting containing gastric juice, loose stool/diarrhea, prone position and relaxation of the lower eyelids.

2.6.6.3 Repeat-dose toxicity

Study Title: Two-Week Repeated Dose Oral Toxicity Study of RU-0211 in Rats.

Study no. 500915

Conducting laboratory (and location if not Sponsor): ____

Dates of study initiation & completion: March 18, 1996 & March 21, 1997

GLP compliance: No

QA Report Yes ( ) No (X)

Methods: The study was conducted in — CD Sprague-Dawley rats (5 animals/sex/group). RU-0211 was administered by oral gavage at doses of 0.008, 0.04, 0.2, 1 and 5 mg/kg doses for 2 weeks.

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot# 4.

Formulation/vehicle: Distilled water containing 1% Polysorbate 80.

Times at which Observations were made:

Clinical signs- The animals were observed twice daily for clinical signs and mortality.

Body weights- Body weights were measured twice a week during the dosing period.

Food and water consumption- Food and water consumption was measured once a week.

Hematology- Blood samples for hematology examinations were collected from the surviving animals at the end of the dosing period.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected at the end of the dosing period.

Urinalysis- Not done.

Gross pathology- At the end of the dosing period, surviving animals were sacrificed and complete necropsies performed.

Organs weighed- The weights of the following organs were recorded.

Brain, pituitary, salivary gland, thyroid, thymus, lung, heart, liver, spleen, kidney, adrenal, testes, prostate, ovary and uterus.

Histopathology- Following organs for all animals were fixed in fixatives for microscopic examinations. Histopathological examinations of all organs were conducted only of the control and the high dose group. The stomachs of animals from all groups were examined histologically. As abnormalities in the bone and

bone marrow were observed in the 5 mg/kg group, all animals of the 0.2 mg/kg group were examined histologically.

Heart, aorta, lung, trachea, liver, pancreas, tongue, salivary gland, alimentary canal (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), thymus, spleen, lymph node, kidney, urinary bladder, testes, epididymis, seminal vesicle, prostate, ovary, uterus, vagina, mammary gland, pituitary, thyroid, parathyroid, brain, spinal cord, skin, eye ball, Harderian gland, bone and bone marrow.

Toxicokinetics- Not conducted.

Results:

Clinical signs: One female from the 5 mg/kg group died on the day of scheduled necropsy. This animal showed abnormal clinical signs that included loose stool or diarrhea, decreased locomotor activity, salivation, abdominal distention and soiled fur. Loose stool, decreased locomotor activity and salivation were observed in animals receiving 1 and 5 mg/kg doses.

Body weights: The body weights of the control male and female animals before initiation of dosing were 234.2±12.0 and 156.0±5.3 g respectively. The weights of the males and females at the end of the dosing period were 331.8±13.9 and 191.8±12.6 g respectively. Decreases in body weights (9.1 to 22.4%) were observed in males receiving 1mg/kg and higher doses from Day 4 of dosing. No changes in body weights were observed in females.

Food Consumption: The food consumption of the control males and females on day 3 of dosing were 26.0±2.1 and 15.6±1.3 g/day/animal. Males receiving the 1 mg/kg or higher doses had decreased food consumption on all measuring days (40% to 80%). Females receiving the 5 mg/kg dose had decreased food consumption (49% to 55%).

Hematology: Males receiving the 1 mg/kg dose and higher doses had decreased platelet count (31.3% to 53%), and prolongation of PT (10% to 11%). In females, decreased hematocrit (6.1%), decreased lymphocyte count (23.4%) and increased neutrophil count (285%) were observed at the 5 mg/kg dose

Clinical chemistry: Male animals receiving 1 mg/kg and higher doses had decreased total protein levels (11.5% to 18.2%), and high dose males had increased AST (127%), decreased glucose (27.6%), decreased potassium (15%) and decreased inorganic phosphorus (20.2%) levels. Females receiving 1 and 5 mg/kg doses of RU-0211 increased AST levels (45.7% and 157.7%, respectively) and decreased total protein (10.8% to 20.8%) levels. High dose females had increased alkaline phosphatase (54.9%) and decreased glucose (20.8%) levels.

Gross Pathology: Males receiving the high dose had decreased size of the thymus, spleen, seminal vesicles and prostate, enlarged adrenal gland and distention of the alimentary gland. High dose females had decreased size of the thymus, enlarged adrenal gland and distention of the alimentary gland. The gross pathological changes observed in male and female rats are shown in the Tables below.

Table 12. Necropsy finding of dead female rats in repeated dose toxicity study of RU-0211 by oral administration for 2 weeks

Group	Control	RU-0211				
(mg/kg)	0	0.008	0.04	0.2	1	5
Number of females	0	0	0	0	0	1
Normal	-	-	-	-	-	0
Thymus						
Small	-	-	-	-	-	1
Spleen						
Small	-	-	-	-	-	1
Adrenal(bilateral)						
Large	-	-	-	-	-	1
Alimentary canal						
Distention	-	-	-	-	-	1

Table 13. Necropsy finding of surviving female rats in repeated dose toxicity study of RU-0211 by oral administration for 2 weeks

Group	Control	RU-0211				
(mg/kg)	0	0.008	0.04	0.2	1	5
Number of females	5	5	5	5	5	4
Normal	5	5	5	4	4	0
Thymus						
Small	0	0	0	0	0	4
Adrenal(bilateral)						
Large	0	0	0	1	1	3
Stomach(forestomach mucosa)						
Diffuse dark red spots	0	0	0	0	0	1
Alimentary canal						
Distention	0	0	0	0	0	4

Organ weight: Males receiving 1 and 5 mg/kg doses had decreased thymus (76.5% and 500%, respectively) and kidney (12.7% and 18.5%, respectively) weights (relative to body weight) and increased adrenal weights (relative, 64.5% and 83.3%, respectively). High dose males had increased brain weight (21.5%, absolute), and decreased heart (25.8%, relative), spleen (41.7%, relative) and prostate (42.0%, absolute) weights. Females receiving 1 and 5 mg/kg doses had increased adrenal weights (absolute, 28.3% and 35.9%, respectively), and high dose females had decreased pituitary (29.6%), thymus (82.7%) and spleen (42.6%) weights (relative).

Histopathology: Histopathological examinations of all tissues were conducted only of the control and the high dose groups. Males receiving the high dose had clear cell changes in the hepatocytes (3/5), atrophy of the thymus (4/5), decreased cellularity of the splenic white pulp (1/5), blood resorption of mesenteric lymph node (4/5), fibrous osteodystrophy of the femur (5/5, mild to moderate), fibrous osteodystrophy of the sternum (5/5, mild) and decreased cellularity of the bone marrow of the femur and sternum (5/5). High dose females had clear cell changes in the liver cells (2/4), atrophy of the thymus (4/4), blood resorption of the mesenteric lymph node (4/4), fibrous osteodystrophy of the femur and sternum (4/4,

slight to mild) and decreased cellularity of the bone marrow (2/4). No changes in the bone were observed at 0.2 mg/kg; however, the bone marrow of the 1 mg/kg animals were not examined histologically.

Toxicokinetics: Not conducted.

Summary: In the 2 –week oral-gavage toxicity study with RU-0211 in rats, the drug was administered at doses of 0.008, 0.04, 0.2 and 1 and 5 mg/kg. There was mortality at the 5 mg/kg dose. Loose stool and diarrhea were observed at 1 and 5 mg/kg doses. The target organs of toxicity were the liver (clear cell changes in the hepatocytes), thymus (atrophy), spleen (decreased cellularity of the white pulp), mesenteric lymph nodes (blood resorption), and bone and bone marrow (fibrous osteodystrophy and decreased cellularity of the bone marrow). The no effect dose was 0.04 mg/kg.

Study Title: Four-Week Oral Toxicity Study with RU-0211 in Rats with a 4-Week Recovery Period.

Sponsor's ID # 500116

Conducting laboratory (and location if not Sponsor):

Dates of study initiation & completion: March 18, 1996 & January 7, 1998

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Methods:

The sponsor stated that the doses for this 4-week oral toxicity study were based on a preliminary study in which rats received repeated oral doses of RU-0211 (0.008, 0.04, 0.2, 1 and 5 mg/kg) for 2 weeks. Deaths were observed at 5 mg/kg and there were abnormal hematology, blood chemistry, gross pathology and histopathological findings at this dose. So, the sponsor decided to use 1 mg/kg as the highest dose in the 4-week toxicity study. The low and the medium doses were selected as 0.04 ($1/25^{th}$ of the high dose) and 0.2 ($1/5^{th}$ of the high dose) mg/kg/day respectively.

Dosing information:

Species	CD Sprague-Dawley (SPF) rats
#/sex/group or time point	10/sex/group
Age	4 weeks
Weight	Males 75-86 g; Females 69-81 g
Satellite groups used for toxicokinetics or recovery	**Satellite groups-** 6/sex/group were used for toxicokinetic studies; **Recovery-** 6/sex/group in control and high -dose groups were used for 4-week recovery.
Dosage groups in administered units	0, 0.04, 0.2 and 1 mg/kg/day
Route, form, volume and infusion rate (if i.v.)	RU-0211 was dissolved in distilled water containing Polysorbate 80 (1%); final drug concentrations were 0 0.04 and 0.2 mg/ml. The drug was administered by oral gavage for 28 days (5 ml/kg).

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot # 5.

Formulation/vehicle: Distilled water containing 1% Polysorbate 80.

Times at which Observations were made:

Clinical signs- Twice daily during the dosing period and once a day during the recovery period.

Body weights- Twice a week during the dosing period and once a week during the recovery period.

Food and water consumption- once a week during the dosing and recovery periods.

Ophthalmoscopy- once a week before initiation of dosing and during the dosing and recovery periods. The fundus was examined with a fundus camera and the fundus of 3 males and 3 females from each group were photographed on day 1 and week 4 of dosing.

EKG- Not done

Hematology- at the end of the dosing and recovery periods.

Clinical chemistry- at the end of the dosing and the recovery periods.

Urinalysis- Week-4.

Gross pathology- At the end of the dosing or recovery periods respectively.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, salivary gland, spleen

Histopathology- Histopathological examinations of the following organs were conducted only in the control and the high dose groups.

Adrenals, aorta, bone marrow, bone, brain, cecum, colon, epididymis, esophagus, eye, Harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular), mammary glands, ovary, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina.

As treatment related abnormalities were observed in the adrenals of the high dose groups, histopathological examinations were also conducted in the 0.2 mg/kg males and 0.04 and 0.2 mg/kg females.

Toxicokinetics- For toxicokinetic analysis, blood samples were withdrawn from the satellite animals at 15, 30 and 60 minutes after dosing on days 1, 15 and 28 of the dosing period.

Results:

Clinical signs: There were no deaths in any groups. Loose stool (from days 9-10) and abdominal distention (from days 12-13) were observed in males and females of the 1 mg/kg group. The number of animals showing the above signs increased with continued dosing; at the end of the dosing period, loose stool was observed in all males and females and abdominal distention in almost half of the animals. Dose-dependent salivation was observed in both males and females receiving RU-0211. Loose stool and abdominal distention were also observed in the high dose males (up to 2 weeks after withdrawal) and females (up to 1 week after withdrawal) during the recovery period.

Body weights: The body weights of the control male and female animals before initiation of dosing were 184.4±4.9 and 149.2±6.1 g respectively. The weights of the males and females at the end of the dosing period were 355.4±25.3 and 219.0±15.7 g respectively. Decreased body weight gains (9.23 to 11%) were observed in the 1mg/kg males from Day 4 to Day 28 of dosing. On the other hand, the females receiving the 1 mg/kg dose had increased body weight gains, beginning from Day 15 of dosing. The body weight gains of the males receiving the 1 mg/kg dose were also lower than control on Days 1 (9.3%) and 8 (7.5%) of the recovery period.

Food and water consumption: The food consumptions of the control males and females on day 3 of dosing were 22.3±1.1 and 17.4±1.7 g/day/animal. Males and females receiving the 1 mg/kg dose had significantly lower food consumption on Day 3 of dosing (34.5% and 41.9% respectively). Afterwards, a significant increase in the food consumption was observed in the males on Day 24 (19.3%) and in the females on Day 17 (12.2%) of dosing.

A significant increase in the food consumption was observed on Day 3 of the recovery period in both males (30%) and females (17.6%) receiving the 1 mg/kg dose.

Water consumption was higher than controls in both males (9.3%, 17.1% and 36.1% respectively at 0.04, 0.2 and 1 mg/kg on day 10) and females (24.7% and 62.1% respectively at 0.2 and 1 mg/kg on day 10) during the dosing period. The high dose males (29.2% and 31.4% respectively) and females (55.6% and 53.3% respectively) had significantly increased water consumption also on days 17 and 24.

Significantly decreased water consumption was observed in both males (21.5%) and females (22.4%) of the 1 mg/kg group on Day 3 of dosing. During the recovery period, there was an increase in water consumption in the 1 mg/kg males on Day 3 (39.7%) and in the 1 mg/kg females on Days 3 (33.6%) and 10 (26.6%).

Ophthalmoscopy: No ophthalmologic abnormalities were observed in any groups.

Electrocardiography: Not done.

Hematology: The hematological parameters of the control male and female animals are given in the table below.

WBC $X10^3/mm^3$	RBC $X10^6/mm^3$	Hemoglo-bin (g/dl)	Hematocrit (%)	MCV (μm^3)	MCHC (g/dl)	Platelets $X10^4/mm^3$	Fibrinogen (mg/dl)	Reticulo-cytes (%)	PT (sec)	APTT (sec)
Males										
64.1±16.7	706.0±124	13.88±1.34	41.17±7.06	58.41±1.87	34.44±4.85	91.37±17.8	267.0±19.8	28.5±5.9	14.8±1.07	29.24±2.18
Females										
79±26.4	790.8±32.8	14.18±0.56	42.8±1.86	54.12±1.51	33.15±0.27	95.53±11.4	220.7±20.9	29.2±2.8	15.78±0.35	28.72±2.19

Males receiving the 0.04 mg/kg dose had higher WBC levels (46.95%) and the males and females receiving the 1 mg/kg dose had higher fibrinogen (10.86% in males, 19.76% in the females) and WBC (42.75% in males, 60.37% in females) levels. However, these changes were not dose-related. No changes were observed in the recovery animals.

Clinical chemistry: The male animals receiving RU-0211 had slightly decreased triglycerides (control, 75.66±11.26 mg/dl; 13.1%, 17.4% and 17.8% decreases at 0.04, 0.2 and 1 mg/kg doses respectively) and potassium (control, 4.95±0.24 mEq/l; 6.9%, 6.1% and 11.5% decreases at 0.04, 0.2 and 1 mg/kg doses respectively) levels. The females had decreased total protein (control, 5.69±0.44 g/dl; 6.9% and 11.1% decreases at 0.2 and 1 mg/kg doses) and albumin (control, 3.376±0.322 g/dl; 9.2% and 15.4% decreases at 0.2 and 1 mg/kg doses) levels.

At the end of the recovery period, females receiving the 1.0 mg/kg dose had lower total protein (11.68%), albumin (13.3%) and calcium (4.6%) levels.

Urinalysis: At the end of the dosing period, there were significant increases in the urinary volume (42.4%) and decrease in K^+ levels (39.4%) in the females receiving the 1 mg/kg dose. At the end of the 4-week recovery period, significant increases in the urinary volume (42%), decreases in the specific gravity, Na^+ (25.1%), K^+ (23.6%) and Cl^- (25.6%) levels were observed in the 1 mg/kg females. No significant changes were observed in the male animals receiving RU-0211 for 4 weeks.

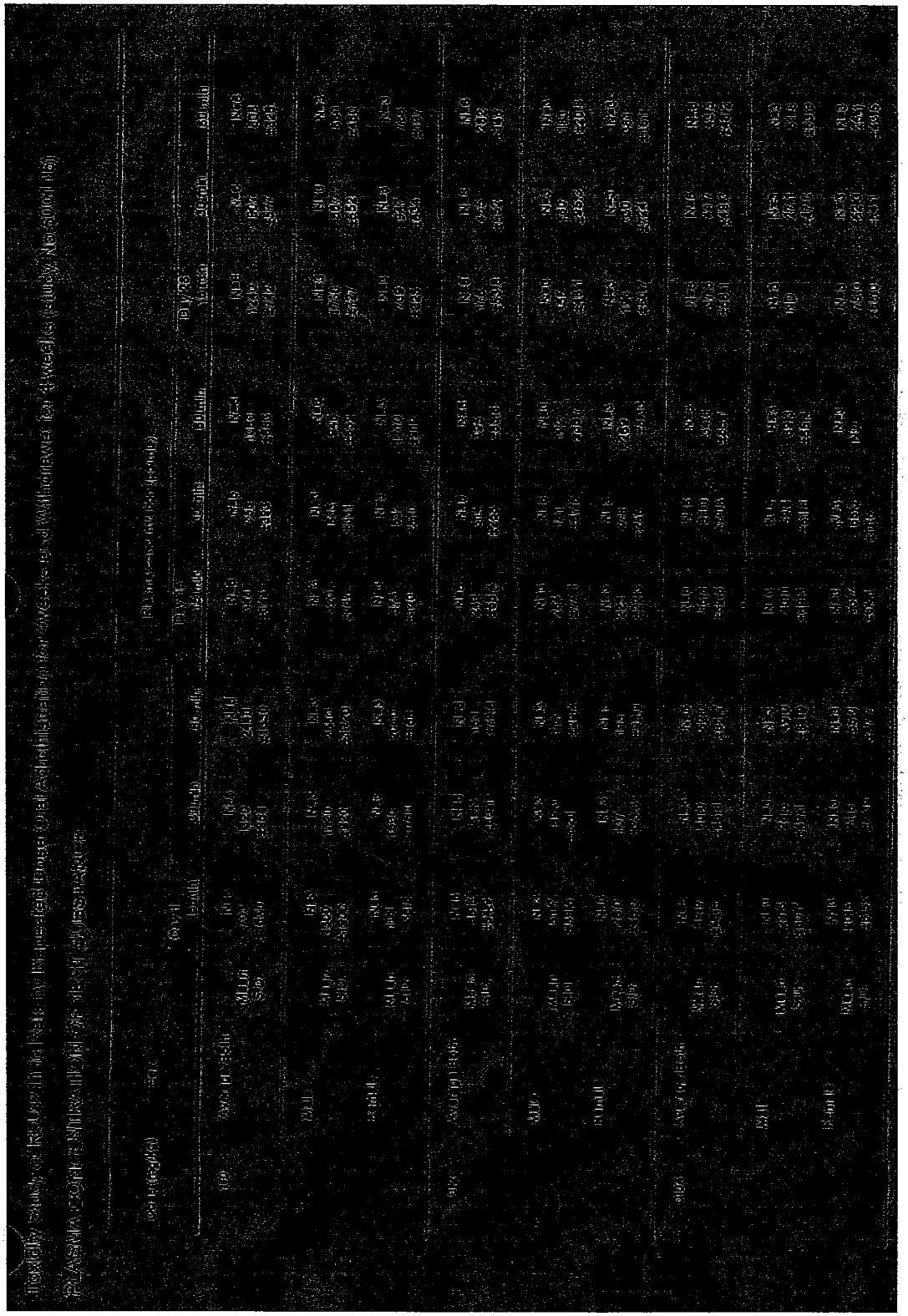
Organ weight: The absolute weights of the salivary glands (13.5%; $p<0.01$) and testes (10.1%; $p<0.01$) of the males receiving the 0.04 mg/kg dose were higher than controls. Males receiving the 1.0 mg/kg dose had decreased thymus weights (absolute, 27.8% and relative, 24.8%; $p<0.01$) and increased adrenal weights (absolute, 40.3% and relative, 45.8%; $p<0.05$). The females receiving the high dose had increased adrenal weights (absolute, 16.4% and relative, 15.7%; $p<0.05$), increased ovary weight (absolute, 15.7%; $p<0.05$) and increased liver weights (absolute, 19.8% and relative, 8.1%; $p<0.01$).

At the end of the recovery period, the high dose males had higher adrenal weights (absolute, 13.4%; $p<0.05$ and relative, 15.3%; $p<0.01$) and the high dose females had higher ovary weights (absolute, 29.3% and relative, 35.0%; $p<0.05$).

Gross pathology: Bilateral adrenal enlargement was observed in 7 (of 10) males and 7 (of 10) females of the 1 mg/kg group, and distention of the alimentary canal was observed in 10 (of 10) males and 8 (of 10) females in the same group. At the end of the recovery period, one male in the 1 mg/kg group had a bulla in the pituitary.

Histopathology: Slight to mild hyperplasia of the zona fasciculata of the adrenals was observed in the females receiving 0.04 mg/kg and higher doses (1 of 10 in 0.04 mg/kg, 3 of 10 in 0.2 mg/kg and 6 of 10 in 1 mg/kg) and the males receiving the 1 mg/kg dose (8 of 10 animals). One female of the 1 mg/kg group had mild atrophy of the thymus cortex. Mineralization of the germinal center of the lymphatic nodule of the ileum was observed in 3 males and 1 female of the 1 mg/kg group. At the end of the 4-week recovery period, no treatment related changes were observed.

Toxicokinetics: Following oral administration of RU-0211 to rats, mean plasma concentrations of the parent compound increased with increasing doses in both males and females. There were no significant differences in plasma drug concentrations between males and females. However, the individual plasma drug concentration values were variable. There was no accumulation of the drug during the 28-day administration period in the male and female animals. The mean plasma RU-0211 concentrations of the male and female rats at different times during the 28-day administration period are shown in the sponsor's Tables below.



Key Study Observations: In the 4 –week oral-gavage toxicity study with RU-0211 in rats, the drug was administered at oral doses of 0.04, 0.2 and 1 mg/kg doses to male and female animals. Loose stool and abdominal distention was observed in both males and females receiving the high dose. Mineralization of the germinal center of the ileum was observed in males and females receiving the high dose. Enlargement and increased weight of the adrenals was observed in males and females receiving the 1 mg/kg dose, and histopathological examination revealed hyperplasia of the zona fasciculata in females at all doses and in the high dose males. The target organ of toxicity was the adrenal gland and the gastrointestinal tract, and the no adverse effect level (NOAEL) was 0.04 mg/kg in the females and 0.2 mg/kg in the males.

Study Title: Repeated Dose Toxicity Study of RU-0211 by 26-Week Oral Administration in Rats.

Study Report No: RTU/SR00-010 (Cross reference number: Study No. 550218)

Conducting laboratory and location:

Date of study initiation: **April 13, 1999**

Date of study report: March 3, 2000 (Pre-Final Report)

GLP compliance: This study was conducted in accordance with the Guidelines for Toxicity Studies of Drugs [Revision of the Guidelines for Single/Repeated Dose Toxicity Studies (Notification No. 88 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, August 10, 1993)]. The study was conducted in compliance with GLP standards specified in the Japanese Ministry of Health and Welfare Ordinance No. 21 (March 26, 1997).

QA- Report Yes ( ) No ( ) (Unknown, Pre-Final Report)

Methods: In a 26-week oral toxicology study, rats received RU-0211 at doses of 0.016, 0.08, and 0.4 mg/kg/day.

Dosing:

- **species/strain:** CD (SD) IGS strain rats (SPF)
- **#/sex/group or time point:** 10 rats/sex/group
- **age:** Rats were approximately 6 weeks old at the start of treatment.
- **weight:** On day 1 of treatment, male and female rats had mean body weights of 190-191 g and 157 g, respectively.
- **satellite groups used for toxicokinetics or recovery:** 3 rats/sex/group for toxicokinetics. (Note: When blood was collected from satellite groups on day 1 for measurement of blood drug levels, red blood cells were inadvertently discarded. The 3 initial satellite groups were subsequently removed from the study and replaced with 3 new satellite groups for measurement of blood drug levels).
- **dosage groups in administered units:** 0.016, 0.08, and 0.4 mg/kg/day
- **route, form, volume, and infusion rate:** RU-0211 or vehicle was administered by oral gavage using a dose volume of 5 mL/kg.

Drug, lot#, radiolabel, and % purity: RU-0211, Lot 9

Formulation/vehicle: 1% aqueous solution of polysorbate 80

Observations and times:

- **Clinical signs:** Animals were observed for clinical signs of toxicity and mortality twice per day, before and after dosing.
- **Body weights:** Body weight was measured twice per week through 5 week of treatment and once per week, thereafter.
- **Food and water consumption:** Food and water consumption were measured once per week (food and water intake were measured for 2 consecutive days and daily food and water consumption were calculated).
- **Ophthalmoscopy:** For all animals, the anterior of the eye was grossly observed prior to start of treatment and at the completion of the treatment period. After dilation with a mydriatic agent, the fundi oculi were examined using a retinal camera and abnormal fundi oculi were photographed. Fundi oculi of 5 rats/sex/group were also photographed on the first day of treatment and prior to completion of the treatment period.
- **EKG:** Not performed.
- **Hematology:** Blood for measurement of hematology parameters was collected from the abdominal aorta on the day after final treatment.
- **Clinical chemistry:** Blood for measurement of blood biochemistry parameters was collected from the abdominal aorta on the day after final treatment.
- **Urinalysis:** Urine for analysis was collected over a 24 hr period from all animals prior to completion of the treatment period. Urine was collected for 3 hr while animals were being fasted with water available ad libitum. Urine was collected for 21 hr with both food and water available ad libitum.
- **Gross pathology:** All animals were necropsied on the day after the final treatment.
- **Organs weighed:** Absolute and relative organ weights were determined for the brain (cerebrum, cerebellum, and medulla oblongata), hypophysis, salivary glands (submandibular glands and sublingual glands), thyroid glands (including the parathyroid glands), thymus, lungs (bronchus included), heart, liver, spleen, kidneys, adrenal glands, male reproductive organs (testes and prostate), and female reproductive organs (ovaries and uterus).
- **Histopathology:** Organs and tissues were collected and preserved as follows: eyes, heart, aorta, lungs, trachea, liver, pancreas, tongue, salivary glands (sublingual glands and submandibular glands), digestive tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), thymus, spleen, lymph nodes (mesenteric), kidneys, urinary bladder, male reproductive organs (testes, epididymides, seminal vesicle, and prostate), female reproductive organs (ovaries, uterus, and vagina), mammary glands, hypophysis, adrenal glands, thyroid glands, parathyroid glands (when available), brain (cerebrum, cerebellum, and medulla oblongata), spinal cord, Harderian glands, bone (femur and sternum), bone marrow (femur and sternum), auricles (when gross abnormalities were present at necropsy), and skin (when hair loss was noted). The organs and tissues from the control and high dose groups were embedded in paraffin, stained with hematoxylin and eosin, and submitted to histopathological examination. Stomachs from all treatment groups were submitted to histopathological examination.
- **Toxicokinetics:** Blood for measurement of drug levels was collected on day 1 of treatment at 15, 30, and 60 min after dosing. Blood was also collected for measurement of drug levels during weeks 9 and 18 and on the final day of dosing. Plasma and red blood cells were collected and sent to Ueno Institute for Medical Sciences for measurements of drug levels.
- **Other:** None.

Results:

- **Clinical signs:** Salivation was observed immediately after dosing for all male and female treatment groups. For male and female rats at 0.016 mg/kg/day, salivation was first observed on days 31 and 30, respectively, and was observed for 1-5 rats/sex/day, thereafter. For male and female rats at 0.08 mg/kg/day, salivation was first observed on days 23 and 22, respectively, and was observed for ≥5 rats/sex/day, thereafter. For male and female rats at 0.4 mg/kg/day, salivation was first observed on days 12 and 11, respectively, and was observed for ≥6 rats/sex/day, thereafter.

- **Mortality:** There were no deaths during the treatment period.

- **Body weights:** A slight suppression of final body weight (91.9% of the control) and body weight gain (87.6% of the control) for male rats at 0.4 mg/kg/day was observed.

Final body weights and body weight (BW) gain for rats that RU-0211 at doses of 0, 0.016, 0.08, and 0.4 mg/kg/day for 26 weeks.

Parameter	Male rats				Female rats			
	0	0.016	0.08	0.4	0	0.016	0.08	0.4
BW, day 1 (g)	190	190	190	191	157	157	157	157
BW, day 183 (g)	609	653	648	560	335	335	325	324
% of Final Control BW	100	107.2	106.4	91.9	100	100	97	96.7
BW Gain, % of Control	100	110.5	109.3	87.6	100	100	107	93.8

- **Food consumption:** There were no treatment-related effects on food consumption. Water consumption was increased for male treatment groups and female rats at 0.4 mg/kg/day. Water consumption for male rats at 0.016, 0.08, and 0.4 mg/kg/day was increased to 126.6, 133, and 148.8% of the control (30.27 mL/day), respectively. Water consumption for female rats at 0.4 mg/kg/day was increased to 114.3% of the control (29.35 mL/day).

- **Ophthalmoscopy:** There were no treatment-related ophthalmic effects.

- **Hematology:** White blood cell counts for male rats at 0.016, 0.08, and 0.4 mg/kg/day were increased to 119.6, 152.9, and 139.2% of the control (51 x $10^2/\mu L$), respectively. White blood cell counts for female rats at 0.4 mg/kg/day were increased to 113.2% of the control (38 x $10^2/\mu L$). Red blood cell counts for female rats at 0.08 and 0.4 mg/kg/day were decreased to 95.1 and 94.4% of the control (772 x $10^4/\mu L$), respectively. Mean corpuscular volume for male rats at 0.4 mg/kg/day were increased to 103.6% of the control (52.8 fL). Mean corpuscular hemoglobin levels for male treatment groups were increased to 101.7-104.6% of the control (17.6 pg). Mean corpuscular hemoglobin levels for female rats at 0.08 and 0.4 mg/kg/day were both increased to 103.2% of the control (18.8 pg). Prothrombin time and activated partial thromboplastin time for female rats at 0.4 mg/kg/day were increased to 106.2 and 106.7% of controls (14.5 and 22.5 sec), respectively. Fibrinogen levels for female rats at 0.08 and 0.4 mg/kg/day were increased to 108.5 and 109.8% of the control (177 mg/dL), respectively.

- **Clinical chemistry:** Total cholesterol levels for male and female rats at 0.4 mg/kg/day were decreased to 73.6 and 75.9% of controls (93.7 and 87.4 mg/dL), respectively. Total protein levels for male and female rats at 0.4 mg/kg/day were decreased to 96.6 and 91.2% of controls (5.9 and 6.8 g/dL), respectively. The α_1-globulin percentage for male rats at 0.4 mg/kg/day was decreased to 90.4% of the control (24.0%). γ-Globulin percentages for male rats at 0.08 and 0.4 mg/kg/day were increased to 118.4 and 136.8% of the control (3.8%), respectively. The albumin concentration and percentage for female rats at 0.4 mg/kg/day were decreased to 86.8 and 94.8% of controls (3.80 g/dL and 56.2%), respectively. The α_2-globulin and γ-globulin percentages for female rats at 0.4 mg/kg/day were increased to 117 and 139% of controls (5.3 and 4.1%), respectively. The albumin/globulin ratio for female rats at 0.4 mg/kg/day was decreased to 89% of the control (1.29). Total bilirubin levels for female rats at 0.4 mg/kg/day were increased to 150% of the control (0.08 mg/dL). Calcium levels for female rats at 0.4 mg/kg/day were decreased to 95.1% of the control (10.3 mg/dL).

- **Urinalysis:** Several changes were observed in urinalysis parameters; however, no histopathological lesions were observed in the kidneys. Changes in urinalysis parameters were most likely due to drug-induced dehydration and subsequent increased water consumption. Ketone bodies for male rats at 0.4 mg/kg/day (4-negative, 6-slight) were increased as compared to the control (8-negative, 2-slight). Ketone bodies for female rats at 0.08 mg/kg/day (7-negative, 3-slight) and 0.4 mg/kg/day (5-negative, 5-slight) were increased as compared to the control (10-negative, 0-slight). Urobilinogen levels for male rats at 0.4 mg/kg/day (7-negative, 3 at 1 mg/dL) were increased as compared to the control (10-negative). Protein levels were increased for female rats at 0.08 mg/kg/day (1-negative, 1 at 10-20 mg/dL, 3 at 30 mg/dL, 4 at 100 mg/dL, and 1 at 1000 mg/dL) and 0.4 mg/kg/day (0-negative, 1 at 10-20 mg/dL, 4 at 30 mg/dL, and 5 at 100 mg/dL) as compared to the control (2-negative, 7 at 10-20 mg/dL, and 1 at 30 mg/dL). Potassium levels for male and female rats at 0.4 mg/kg/day were decreased to 46.2 and 60.3% of controls

Organ	Male rats				Female rats			
	0	0.016	0.08	0.4	0	0.016	0.08	0.4
Stomach -thickening of limiting ridge	0	5	10	10	0	0	10	10

- **Histopathology:** The stomach was the target organ of toxicity. Proliferation of epithelial basal cells in the limiting ridge of the stomach was observed for all male and female treatment groups.

Histopathological findings for rats that received RU-0211 at oral doses of 0, 0.016, 0.08, and 0.4 mg/kg/day for 26 weeks.

Organ	Male rats				Female rats			
	0	0.016	0.08	0.4	0	0.016	0.08	0.4
Stomach -proliferation/ down growths, basal cells, limiting ridge	0	5	8	9	0	6	6	10

Toxicokinetics:

The plasma drug concentrations increased with increasing dose at all time points in both males and females. The plasma drug concentrations appeared to be higher in females than that in males in most part of the dosing period (except in weeks 8 and 18 at 0.04 mg/kg). The plasma RU-0211 concentrations at different times of dosing in male and female rats are summarized in the Table below.

Table: Plasma concentrations of RU-0211 at 60 minutes during 26-week daily administration of different doses of in rats (pg/ml).

Dose (mg/kg)	Day 1	Week 8	Week 18	Week 28
0.04				
Male	573±493	740±277	461±161	249±137
Female	592±33	569±451	408±98	757±399
0.08				
Male	23.6±40.9	28.1±48.7	31.8±55.0	49.1±59.4
Female	49.9±46.9	56.7±49.1	94.1±107	131±68.9
0.016				
Male	ND	ND	ND	ND
Female	23.7±41.0	16.8±29.1	23.4±40.6	43.9±39.1

ND, not detectable

Key Study Findings: In a 26-week oral toxicology study, rats received RU-0211 at doses of 0, 0.016, 0.08, and 0.4 mg/kg/day. The dose of 0.08 mg/kg/day could be considered a tolerated dose. Salivation immediately after dosing was observed in all male and female treatment groups. Final body weights (92% of control) and body weight gains (87.6% of control) were slightly suppressed for male rats at 0.4 mg/kg/day. Water consumption was increased for all male treatment groups and female rats at 0.4 mg/kg/day. The stomach was the target organ of toxicity. Proliferation of

epithelial basal cells in the limiting ridge of the stomach was observed for all male and female treatment groups.

"Thirteen (13)-Week Oral Gavage Preliminary Carcinogenicity Study with RU-0211 in Mice"

(Study # YK-Ca-1001)

(Thirteen (13)-Week Oral Dose Range Finding Study With RU-0211 in Mice)

Testing Laboratory: Ueno Fine Chemicals Industry, Ltd., Ueno Institute of Medical Science
Sanda, Hyogo, Japan

Study Start and Completion Dates: November 10, 1999 and November 30, 2000

GLP and QAU Compliance Statement: The sponsor provided a statement of compliance with GLP and QAU.

Drugs Batch NO: RU-0211, Lot # 9

Animals: Male (5 weeks old, 17.4- 22.5 g)
Female (5 weeks old, 14.7-19.1 g)
- B6C3F1 (SPF) mice.

Methods:

The sponsor stated that the purpose of the study was to obtain information on dose selection for future carcinogenicity study in mice. Initially, the male and female animals were divided into five groups (10/sex/group) and groups 1, 2, 3, 4 and 5 were administered oral doses of 0 (no treatment), 0 (vehicle), 0.01, 0.1 and 1 mg/kg/day of RU-0211 (by oral gavage) for 13 weeks. After Week-4 of dosing, the sponsor decided to add 2 more groups of animals (vehicle and 5 mg/kg/day group) to the study, as no changes in body weights or toxic signs were observed in the 1 mg/kg/day group during the 4-weeks of dosing. The control animals received only the vehicle (— , a mid chain fatty acid triglyceride) throughout the dosing period. In addition, 4 satellite groups of animals (40-animals/sex/dose group) were treated with RU-0211 for toxicokinetic studies. Blood collection for toxicokinetic studies were performed at 15, 30, 60 and 120 minutes after dosing on Day 1 and Day 91 (final day of dosing). The animals were observed twice daily for clinical signs and mortality, and the body weights were taken once a week. Food consumption was measured once a week and the daily food consumption was calculated. For hematological analysis, blood was collected by cardiac puncture on the day after the last dosing, all animals were sacrificed and necropsies performed. The weights of the following organs were recorded:

Brain, pituitary, thymus, heart, lung, liver (with gall bladder), kidneys, adrenals, spleen, prostate, testes and ovaries.

Histopathological examinations of the following organs from the control and the high dose animals were conducted. Because gross abnormalities were observed in the stomach, the stomachs of all treatment groups and vehicle treated animals were examined histologically. In addition to the high dose and vehicle treatment groups, one male of the 0.1 mg/kg/day group that had an enlarged adrenal, was examined histologically.

Brain, pituitary, trachea, heart, lung (with mainstem bronchi), liver, adrenal, spleen, pancreas, urinary bladder, thymus, salivary gland (submandibular gland), thyroid with parathyroid, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, eye, optic nerve, bones with bone marrow (sternum and femur), lymph node (mesenteric), spinal cord (cervical thoracic, lumber), skin, seminal vesicle, prostate, testes, epididymides, ovary, uterus vagina and female mammary glands.

Results:

Clinical signs: Loss of hair was observed in both males and females of all groups, including the control. Loose stool and/or diarrhea were observed in both males and females receiving 1 and 5 mg/kg/day doses. The duration of loose stool/diarrhea was increased with increasing doses; at 1 mg/kg/day dose it was observed from Day 1 to Day 14 in males and from Day 1 to Day 6 in females, and at 5 mg/kg/day, diarrhea/loose stool was observed throughout the dosing period. In addition, in the high dose group, abdominal distention was observed in most males and females (beginning from Day 27 of dosing), and decreased locomotor activity was observed in 2-8 of 10 males/day and 2-6 of 10 females/day from Day 1 until the end of dosing.

Mortality: There was no mortality in any group.

Body weights: The mean body weights of the male and female mice before initiation of dosing were 22.3±1.0 and 18.6±0.9 g and at the end of dosing were 30.0±1.3 and 26.8±1.9 g respectively. Treatment with RU-0211 was not associated with any changes in body weights or body weight gains in the male and female animals.

Food consumption: The mean food consumption of the control male and female animals on the day before initiation of dosing was 4.0±0.7 and 3.5±0.7 g/day respectively. Males receiving the 5 mg/kg/day dose had significantly increased food consumption (11% to 32.6% increases as compared with the vehicle treatment group) during Week 2 to Week 13 of the dosing. Females receiving the high dose had increased food consumption (10.9% to 14.3%) in weeks 6, 9 and 10 and those receiving 0.01, 0.1 and 1 mg/kg/day doses had decreased food consumption (16%, 18% and 20% respectively) in Week 13.

Hematology: The hematological parameters of the control male and female animals are shown in the table below.

WBC $x10^2/\mu L$	RBC $x10^4/\mu L$	Hemoglo-bin (g/dl)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets $x10^4/\mu L$	Reticulo-cytes (%)
Males								
13 ± 7	1137 ± 42	17.4±0.5	57.3±2.3	50.4±0.6	15.3±0.5	30.3±1.2	120.0±10.9	1.8±3.0
Females								
12 ± 5	1173±91	16.9±0.8	54.6±4.7	50.9±0.4	15.8±0.6	31.0±1.3	102.3±12.3	1.4±0.5

Males and females receiving 1 and 5 mg/kg/day doses had significantly decreased reticulocyte levels (25% and 47.4% decreases in males and 29.4% and 47.1% decreases in females respectively) as compared with the vehicle treated group. No other changes were observed in any groups.

Gross pathology: Mild thickening of the limiting ridge of the forestomach and glandular stomach was observed in both males (5 of 10 at 0.1 mg/kg, 10 of 10 at 1 mg/kg and 10 of 10 at 5 mg/kg doses) and females (4 of 10 at 0.1 mg/kg, 8 of 10 at 1 mg/kg and 10 of 10 at 5 mg/kg doses) receiving 0.1 mg/kg/day and higher doses. One male receiving the 5 mg/kg/day dose had a miliary cyst at the limiting ridge of the stomach and another male receiving the 0.1 mg/kg/day dose had enlarged adrenals.

Organ Weights: Males receiving RU-0211 had increased adrenal weights (absolute, 10.3%, 15.4%, 15.4% and 21.9%, and relative, 8.3%, 10.8%, 14% and 27.6% at 0.01, 0.1, 1 and 5 mg/kg respectively; significant only at 5 mg/kg) and decreased prostate weights (absolute, 13.2%; relative 9.1% at the high dose). No changes in the organ weights were observed in the female animals.

Histopathology: Histopathological examinations were conducted only of the control and the high dose animals. Because, gross pathological abnormalities in the stomachs were observed at mid-doses (0.1 mg/kg and higher doses; thickening of the limiting ridge), the stomachs of all RU-0211 treatment groups were examined histologically. In addition, one male of the 0.1 mg/kg/day group that had an enlarged adrenal was examined histologically.

In the stomach, acanthosis was observed in 1 female each in the control (also had edema), 0.01 and 0.1 mg/kg/day doses. In the 1 mg/kg/day dose group, the changes included edema in 2 (of 10) males and 2 (of 10) females, acanthosis in 2 (of 10) males and 5 (of 10) females and hyperkeratosis in 1 (of 10) female. In the 5 mg/kg/day dose group, edema was observed in 9 (of 10) males and 7 (of 10) females, acanthosis in 1 male and keratinic cyst in one male.

In the adrenals, 2 (of 10) control males had subcapsular hyperplasia, one 0.1 mg/kg/day male (that had enlarged adrenal) had moderate subcapsular hyperplasia and 4 (of 10) high dose males had mild swelling of the cortical cells.

Toxicokinetics: Plasma concentrations of RU-0211 increased with increasing doses and the peak plasma concentrations were reached within 30 minutes of dosing. The plasma drug concentrations in Week 13 was not higher than that on Day 1, indicating that there was no accumulation of the drug with continuous dosing. Plasma RU-0211 concentrations in male and female mice at different times after dosing are given in the Table below.

Plasma concentrations (pg/ml) of RU-0211 in male and female mice on Day 1 and Week 13 after oral administration of different doses

Males	**Day 1**				**Week 13**			
	15 min.	**30 min.**	**60 min.**	**120 min.**	**15 min.**	**30 min.**	**60 min.**	**120 min.**
0.01 mg/kg	235±349	209±301	153±151	ND	264±365	185±215	57.2±128	ND
0.1 mg/kg	69.0±154	47±105	196±337	ND	152±341	56.4±126	37.4±83.6	ND
1 mg/kg	910±704	611±335	711±298	421±431	970±742	720±285	405±485	310±342
5 mg/kg	4560±4360	7470±5180	6110±3470	2320±1430	2190±3860	5780±5160	2350±499	834±381
Females								
0.01 mg/kg	42.8±95.7	ND	ND	ND	ND	ND	ND	ND
0.1 mg/kg	161±162	204±247	ND	ND	189±199	229±355	ND	ND
1 mg/kg	1310±1210	14900±29200	939±596	260±39.0	735±640	1420±1020	431±288	126±200
5 mg/kg	5310±1460	9270±108000	6760±4290	3170±1130	1460±1220	3900±1680	1200±1400	1820±1640

ND, not detectable (detection limit, —

In summary, in the 13-week dose range finding study with RU-0211 in mice, the animals received oral doses of 0, 0.01, 0.1 and 1, and 5 mg/kg/day of the drug. Loose stool and/or diarrhea were observed in both sexes receiving 1 and 5 mg/kg/day doses; in addition, abdominal distention and decreased locomotor activity were observed in males and females receiving the high dose. Mild thickening at the limiting ridge of the stomach was observed in both males and females receiving 0.1 mg/kg and higher doses; histopathological examination revealed edema and acanthosis in the stomach of both males and females at 1 and 5 mg/kg/day doses. High dose males (4 of 10) had swelling of the cortical cells of the adrenal glands. The target organs of toxicity were the stomach and the adrenal gland. The 1 mg/kg dose can be considered as the maximum tolerated dose as the changes observed at this dose were related to the pharmacological effects of the compound and only mild changes in the stomach was observed.

Study Title: Two-Week Repeated Dose Oral Toxicity Study of RU-0211 in Dogs.

Study no. 640219

Conducting laboratory (and location if not Sponsor):

Dates of study initiation & completion: March 18, 1996 & March 21, 1997

GLP compliance: No

QA Report Yes ( ) No (X)

Methods: The study was conducted in beagle dogs (2 animals/sex/group) which received RU-0211 at oral doses of 0.04, 0.2 and 1 mg/kg doses.

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot# 5.

Formulation/vehicle: RU-0211 was dissolved in medium chain triglyceride (MCT) and encapsulated into capsules.

Times at which Observations were made:

Clinical signs- The animals were observed twice daily for clinical signs and mortality.

Body weights- Body weights were measured on days 1, 4, 8, 11 and 14 of the dosing period.

Food consumption- Food consumption was measured daily during the dosing period.

Ophthalmoscopy: Ophthalmologic examinations of all animals were conducted pre-dose and at the end of the dosing period.

Urinalysis: Urine samples were collected pre-dose and at week-2 of the dosing period.

Hematology- Blood samples for hematology examinations were collected from the surviving animals at the end of the dosing period.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected at the end of the dosing period.

Gross pathology- At the end of the dosing period, surviving animals were sacrificed and complete necropsies performed.

Organs weighed- The weights of the following organs were recorded.

Brain, pituitary, salivary gland, thyroid, thymus, lung, heart, liver, spleen, pancreas, kidney, adrenal, testes, prostate, ovary and uterus.

Histopathology- Following organs for all animals were fixed in fixatives for microscopic examinations. Histopathological examinations of all organs were conducted from the 0.2 and 1 mg/kg groups. The stomachs of animals from all groups were examined histologically.

Heart, aorta, lung, trachea, liver, pancreas, tongue, salivary gland, alimentary canal (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), thymus, spleen, lymph node, kidney, urinary bladder, testes, epididymis, prostate, ovary, uterus, vagina, mammary gland, pituitary, thyroid, parathyroid, brain, spinal cord, skin, eye ball, Harderian gland, bone and bone marrow.

Toxicokinetics- Not conducted.

Results:

Clinical signs: Abnormal stool (loose stool, diarrhea, watery stool), vomiting and decreased locomotor activity were observed in male and female and female dogs treated with RU-0211. The duration of these clinical signs increased with increasing doses.

Body weights: The body weights of the control male and female animals before initiation of dosing were 9.45 and 9.65 kg respectively. The weights of the males and females at the end of the dosing period were 9.0 and 9.5 kg respectively. One male from the 1 mg/kg group had slightly decreased body weight, when compared with the control (5.4%).

Food Consumption: No treatment-related changes in food consumption were observed in any group.

Ophthalmoscopy: No treatment-related ophthalmologic changes were observed in any animal.

Urinalysis: One male and one female each from the 1 mg/kg group had decreased sodium, potassium and chloride concentration in the urine.

Hematology: No treatment-related hematological changes were observed in any group.

Clinical Chemistry: No significant treatment-related changes in the clinical chemistry parameters were observed in any group.

Gross Pathology: No treatment-related abnormal gross pathological changes were observed in any animal.

Organ weight: No significant changes in the organ weights were observed in any group.

Histopathology: No treatment related changes microscopic changes were observed in any group.

Toxicokinetics: Not conducted.

Summary: In the 2 –week oral (capsule) toxicity study with RU-0211 in dogs, the drug was administered at 0.04, 0.2 and 1 mg/kg doses. Diarrhea, loose stool and vomiting were observed in treatment group animals in a dose-dependent manner. Decreased locomotor activity and lacrimation were observed at the 1 mg/kg dose. No target organ of toxicity was identified, and the no effect dose was not established. The high dose (1 mg/kg) was the tolerated dose.

Study Title: Four-Week Oral Toxicity Study with RU-0211 in Dogs with a 4-Week Recovery Period.

Sponsor's ID # 640316

Conducting laboratory (and location if not Sponsor):

Dates of study initiation and completion: December 05, 1996 and September 02, 1998

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Methods:

The sponsor stated that the doses used in this 4-week oral toxicity study were based on a previous 2-week toxicity study in dogs in which the animals received oral doses of RU-0211 at 0.04, 0.2 and 1 mg/kg/day for 2 weeks. At the 1 mg/kg dose, vomiting and abnormal stools were observed daily; inhibition of body weight gains and changes in urinary electrolytes were also observed at this dose. So, the sponsor decided to use 0.5 mg/kg as the high dose in the 4-week oral toxicity study. The medium and the low doses were selected as 0.07 and 0.01 mg/kg respectively, in a detrimental ratio of about 7.

Dosing information:

Species	Beagle dogs
#/sex/group or time point	3-5/sex/group
Age	5.5-6.5 months
Weight	Males 8.2-9.4 kg; Females 7.2-8.4 kg
Satellite groups used for toxicokinetics or recovery	**Recovery-** 2/sex/group in control and high -dose groups were used for 4-week recovery.
Dosage groups in administered units	0, 0.01, 0.07 and 0.5 mg/kg
Route, form, volume and infusion rate (if i.v.)	The test substance or — (control), encapsulated in gelatin capsules, were administered by oral gavage.

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot # 5.

Formulation/vehicle: RU-0211 was mixed with — and encapsulated in gelatin capsules.

Times at which Observations were made:

Clinical signs- Three times a day during the dosing period and once a day during the recovery period.

Body weights- Once a week during the dosing and recovery periods.

Food and water consumption- Food consumption was measured once a week and water consumption was measured once in 4 weeks.

Ophthalmoscopy- Before initiation of dosing, and at the end of dosing and recovery periods.

EKG- Before the start of dosing and at the end of the dosing and recovery periods.

Hematology- At the end of the dosing and the recovery periods.

Clinical chemistry- At the end of the dosing and recovery periods.

Urinalysis- Before initiation of dosing, during Week 4 of dosing and during Week 4 of the recovery period.

Gross pathology- At the end of the dosing or recovery periods.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, epididymis, gall bladder, heart, kidneys, liver, lungs, ovaries, pancreas, parathyroid, pituitary, prostate, salivary gland, spleen, testes, thymus, thyroid, uterus.

Histopathology- The following organs of the control and treatment group animals were examined histologically.

Adrenals, aorta, bone marrow, bone (femur), brain, cecum, colon, duodenum, epididymis, esophagus, eye, gall bladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, ovaries, pancreas, parathyroid, pharynx, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina.

Toxicokinetics- For toxicokinetic studies, blood was collected from the cephalic veins of all animals at 15, 30 and 60 minutes after dosing (Days 1, 15 and 28 of dosing).

Results:

Clinical signs:

There were no deaths in any groups. Loose stool, diarrhea or watery stool was observed in males and females receiving 0.01 mg/kg and higher doses and vomiting of bubbly gastric juice was observed at 0.07 mg/kg and higher doses (vomiting was observed 1-6 hours after dosing, sometimes 2-3 times a day in some animals). Decreased locomotor activity was observed in both males and females at 0.5 mg/kg, being more frequent in the males. Salivation and lacrimation were observed in both sexes at 0.07 mg/kg and higher doses. Decreased locomotor activity, salivation and lacrimation disappeared within 6 hours of administration of RU-0211. The general signs observed in male and female dogs during the first 2-weeks of dosing are given in the table below.

General signs observed in male and female dogs receiving oral RU-0211 for 4 weeks (total number of animals showing the particular sign in a day)

Males Signs	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14
0.01 mg/kg/day								
Loose stool	0	1	0	2	2	0	0	0
Diarrhea	0	0	0	0	0	0	0	0
Vomiting bubbly gastric juice								
0.07 mg/kg/day								
Loose stool	0	2	2	3	2	0	3	1
Diarrhea	2	0	0	0	0	3	1	2
Watery stool	1	0	1	0	3	0	1	0
0.5 mg/kg/day								
Decreased locomotor activity	3	3	3	3	2	2	2	1
Loose stool	0	2	3	4	4	1	6	1
Diarrhea	2	2	0	1	0	3	0	0
Watery stool	0	1	0	0	0	2	1	0
Vomiting bubbly gastric juice	4	4	5	5	5	5	6	7
Females Signs	**Day 1**	**Day 2**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 14**
0.01mg/kg/day								
Loose stool	1	1	1	0	1	0	0	0
Diarrhea	1	1	0	1	0	0	0	1
0.07 mg/kg/day								
Loose stool	1	1	3	0	1	2	2	1
Diarrhea	0	3	0	0	2	0	0	0
Watery stool	0	0	0	1	0	1	2	1
0.5 mg/kg/day								
Decreased locomotor activity	0	0	0	0	0	0	0	1
Loose stool	1	1	2	2	3	2	1	1
Diarrhea	0	3	0	1	4	2	0	2
Watery stool	2	1	2	0	2	2	2	4
Vomiting bubbly gastric juice	5	5	5	5	6	5	4	7

Body weights: The mean body weights of the control male and female animals before the initiation of dosing were 9.80±0.60 and 8.96±0.96 kg and at the end of the dosing period 10.13±0.85 and 9.17±1.31 kg respectively. No treatment related changes in the body weight or body weight gain was observed in any groups.

Food and water consumption: The control males and females had mean food consumptions of 300.0±0.0 and 283.8±25.1 g/day and mean water consumptions of 829.4±144.6 and 697.8±187.1 ml/day respectively. There were no treatment-related changes in the food or water consumptions in any groups.

Ophthalmoscopy- No ophthalmologic abnormalities were observed in any groups.

Electrocardiography: No treatment related changes in EKG parameters (heart rate, QRS, QT, PR interval and electric potential) were observed in any groups.

Hematology: The hematological parameters of the control males and females are given in the table below.

WBC $X10^3/mm^3$	RBC $X10^6/mm^3$	Hemoglo-bin (g/dl)	Hematocrit (%)	MCV (μm^3)	MCHC (g/dl)	Platelets $X10^4/mm^3$	Fibrinogen (mg/dl)	Reticulo-cytes (%)	PT (sec)	APTT (sec)
Males										
75.2±17.4	711.4±50.1	15.78±1.27	47.36±3.68	66.58±2.52	33.30±0.35	28.72±4.78	214.4±34.5	6.6±3.0	9.54±0.98	16.40±0.67
Females										
84.8±15.7	721.8±46.4	15.86±1.19	48.08±3.20	66.62±2.12	32.98±0.58	27.46±6.18	197.0±33.8	5.4±1.1	9.24±0.84	16.38±1.47

There were slight decreases in RBC (16.54%; $p<0.05$), hemoglobin (16.69%; $p<0.01$) and hematocrit (15.6%; $p<0.05$) levels in the males receiving the 0.07 mg/kg dose. Males receiving the 0.5 mg/kg dose had decreased reticulocyte levels (45.45%; $p<0.01$) and the females in this group had increased fibrinogen levels (32.89%; $p<0.05$). At the end of the recovery period, no significant changes were observed.

Clinical chemistry: Males receiving the 0.5 mg/kg dose had decreased AST levels (17.91%; $p<0.01$); females receiving the same dose had an increase in the total cholesterol (33.6%; $p<0.05$) levels. No significant changes were observed in the control and the high dose groups after the 4-week recovery period.

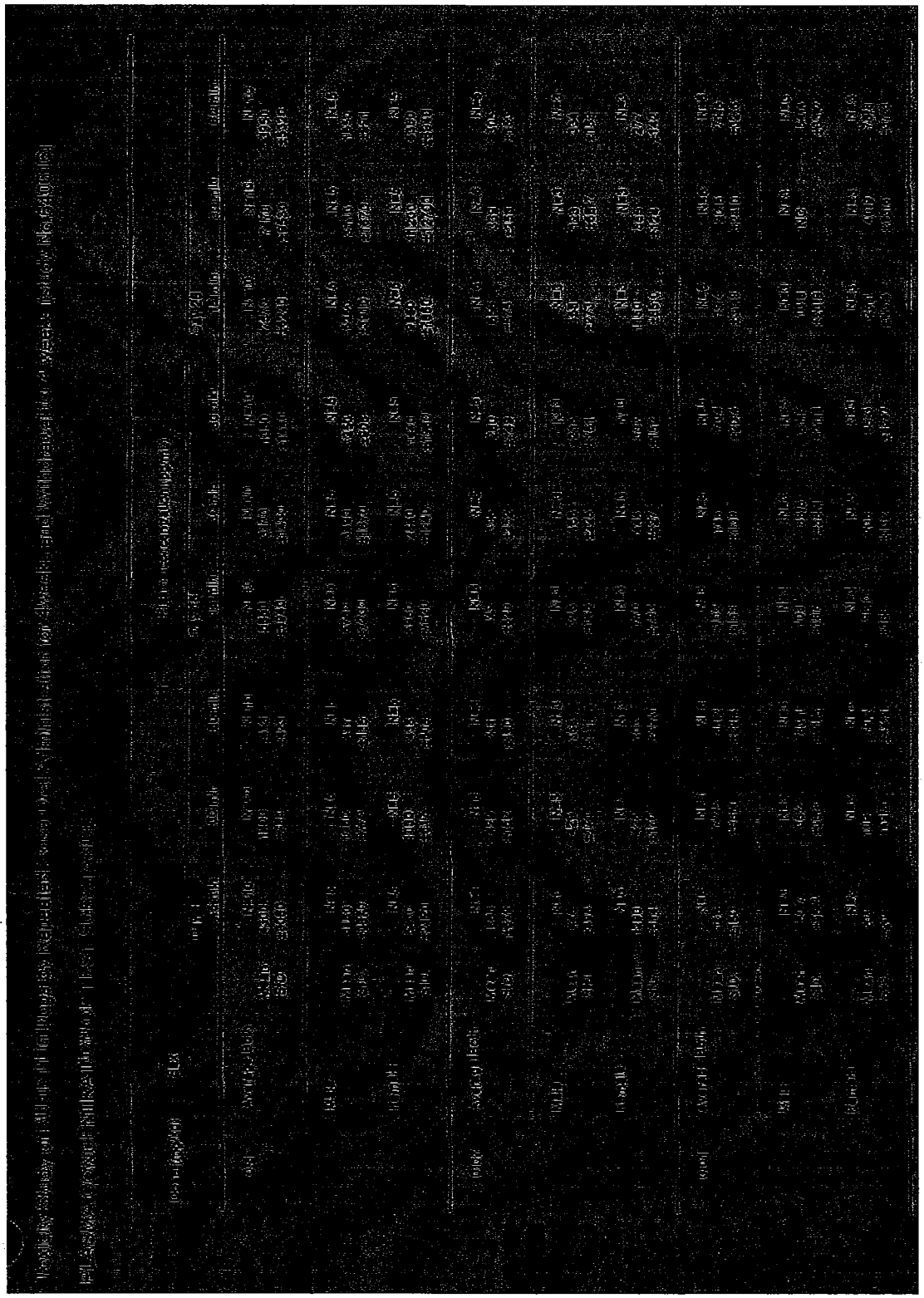
Urinalysis: There were significant and dose-dependent decreases in the urinary concentrations of Na^+ in the males (control, 60.0±22.4 mEq/l; 23.3%, 58.3% and 90.7% decreases at 0.01, 0.07 and 0.5 mg/kg doses respectively). The Cl^- concentration was also lower (67.2%) in the males receiving the high dose. In the females, there were dose-dependent decreases in the urinary Na^+ (control 64.8±31.4 mEq/l; 33.6%, 81.8% and 85.5% decreases at 0.01, 0.07 and 0.5 mg/kg doses respectively) and Cl^- (control, 157.03±22.45 mEq/l; 30.4%, 55.7% and 76.9% at 0.01, 0.07 and 0.5 mg/kg doses respectively) concentrations. Urinary K^+ concentration was also lower in females receiving the high dose (59.2%). At the end of the 4-week recovery period, there were no changes in any parameters.

Organ weight: Males receiving the 0.07 mg/kg dose had higher relative weights of the adrenals (control, 8.07 mg% and treated, 10.40 mg%) and the females in this group had higher submandibular gland weights (relative; control, 0.093 g% and treated, 0.127 g%). In the high dose group, the absolute weights of the pituitary was lower (16.74%) and the relative weights of the adrenals were higher (control, 8.07 mg% and treated, 12.07 mg%) than controls in the males. In the females, submandibular (absolute, 40.62%) and adrenal gland (absolute, 49.93%) weights were higher than controls. At the end of the recovery period, no significant changes were observed.

Gross pathology: Bilateral adrenal enlargement was observed in 1 male (#301) receiving the high dose. Adhesion of the pulmonary lobes to the thoracic wall was observed in 1 male (#101) receiving the low dose. No abnormalities were observed in the animals after the 4-week recovery period.

Histopathology: Slight to mild hyperplasia of the zona glomerulosa was observed in 1 of 3 males and all females (3 of 3) receiving the 0.5 mg/kg dose. Slight microgranuloma of the liver was observed in 1 (of 3) male and all (3 of 3) females of the control group, in 1 (of 3) female of the 0.01 mg/kg group, in 1 (of 3) male and all (3 of 3) females of the 0.07 mg/kg group and in all (3 of 3) males and 1 (of 3) female of the 0.5 mg/kg group. At the end of the 4-week recovery period, slight hyperplasia of the adrenal zona glomerulosa was observed in 1 male receiving 0.5 mg/kg of RU-0211. Slight mineralization of the papilla was observed in 1 control male and all males receiving the 0.5 mg/kg dose. One male in the high-dose group had slight hyperplasia of the thyroid and 1 female in this group had slight lymphoid cell infiltration of the salivary gland.

Toxicokinetics: Following oral administration of RU-0211 to dogs for 28 days, the mean plasma concentrations of the drug increased with increasing doses. There were no significant differences in plasma drug concentrations between males and females. There was no accumulation of the drug during the 28-day administration period in the male and female animals. The mean plasma RU-0211 concentrations of the male and female dogs at different times during the 28-day administration period are shown in the sponsor's Tables below.



Key Study Observations: In the 4 –week oral gavage toxicity study with RU-0211 in dogs, the drug was administered to male and female animals at oral doses of 0.01, 0.07 and 0.5 mg/kg/day. Loose stool, diarrhea and watery stool were observed at all doses in both males and females and the incidences were increased with increasing doses. Vomiting, salivation and lacrimation were observed in both sexes at 0.07 mg/kg and higher doses. There were dose dependent decreases in sodium and chloride concentrations in the urine of both males and females. This could be the secondary to the loss of the electrolytes due to loose stool/diarrhea. Slight to mild hyperplasia of the zona glomerulosa was observed in both males and females receiving the high dose, being prominent in the females. The target organ of toxicity was the adrenal gland and the gastrointestinal tract and the no adverse effect level (NOAEL) was 0.01 mg/kg in both males and females.

Repeated Dose Toxicity Study With RU-0211 by 39-Week Oral Administration in Dogs (Study # 670119)

Testing Laboratory:

Study Start and Completion Dates: April 01, 1999 and December 01, 2000

GLP and QAU Compliance Statement: The sponsor provided a statement of compliance with GLP and QAU.

Drugs Batch NO: RU-0211, Lot # 9

Animals: Male (6.0-6.5 months old, 8.12-9.63 kg)
Female (6.0-7.0 months old, 7.99-8.96 kg)
Beagle dogs.

Methods:

Sixteen male and 16 female Beagle dogs were randomly divided into four groups (4/sex/group). Group 1 animals received the vehicle (mid chain triglyceride) and groups 2, 3 and 4 received oral doses of 0.002 (0.01% w/w), 0.01 (0.05% w/w) and 0.05 (0.25% w/w) mg/kg/day of RU-0211 respectively, administered as oral capsules. The doses were selected on the basis of a 4-week repeated dose toxicity study in Beagle dogs in which male and female animals received 0.01, 0.07 and 0.5 mg/kg/day doses. Loose stool, diarrhea and decreased urinary Na^+ and Cl^- concentrations were observed at 0.01 mg/kg and higher doses, and vomiting was observed at 0.07 mg/kg/day and higher doses. Considering the length of

the dosing period, the sponsor selected 0.05 mg/kg/day as the high dose for the present study and the mid and low doses were selected as 0.01 and 0.002 mg/kg/day respectively (by a factor of 5). The animals were observed for clinical signs and mortality 3 times a day and the body weights were taken once a week. Food consumption was measured daily and the water consumption was measured once a week. Electrocardiography (EKG) examinations were conducted once before initiation of dosing and once a week during weeks 13, 26 and 39 of dosing using standard limb leads (I, II, III, aV_R, aV_L and aV_F). Ophthalmologic examinations were conducted once prior to initiation of dosing and during weeks 13, 26 and 39 of the dosing period. Urinalysis, clinical chemistry and hematological examinations were conducted once before initiation of dosing and in weeks 12, 25 and 38. For toxicokinetic analysis, blood was collected at 15, 30 and 60 minutes after the administration of the first dose, in week 14 and week 27 and on the final day of dosing. All surviving animals were sacrificed on the day after the final dosing and examined macroscopically. The weights of the following organs were recorded.

Brain, hypophysis, submandibular glands, thyroid glands, thymus, lungs, heart, liver (including gall bladder), spleen, kidneys, pancreas, adrenals, male reproductive organs (testes, epididymides and prostate) and female reproductive organs (ovaries and uterus).

Histopathological examinations of the following organs were conducted.

Heart, thoracic aorta, lungs, trachea, liver, gall bladder, pancreas, tongue, pharynx, salivary glands (parotid gland, sublingual glands, submandibular glands), digestive tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum), thymus, spleen, lymph nodes (mesenteric and submandibular), kidneys, urinary bladder, male reproductive organs (testes, epididymides and prostate), female reproductive organs (ovaries, uterus and vagina), mammary glands, hypophysis, adrenal glands, thyroid glands, parathyroid glands, brain (cerebrum, cerebellum and medulla oblongata), spinal cord, sciatic nerve, skin, eyeballs, accessory glands (lacrimal glands), bones with bone marrow (sternum and femur), and skeletal muscle (rectus femoris).

Results:

Clinical signs: Vomiting of bubbly gastric juice, vomiting containing food, loose stool, diarrhea and watery stool were observed in males and females of all groups. The clinical signs observed in the male and female animals are summarized in the table below.

Clinical signs observed (number of total incidences) in the male and female dogs receiving different doses of RU-0211.

Males

Signs

	Control	0.002 mg/kg/day	0.01 mg/kg/day	0.05 mg/kg/day
Vomiting of bubbly gastric juice	32	20	84	239
Vomiting containing food	2	1	3	3
Loose stool	0	12	145	63

Diarrhea	0	1	389	902
Watery stool	0	0	5	21
Females				
Signs				
Vomiting of bubbly gastric juice	32	19	39	234
Vomiting containing food	3	1	1	7
Loose stool	0	13	81	78
Diarrhea	0	3	188	706
Watery stool	0	0	2	12

Mortality: One control male (M01004) had decreased locomotor activity, bubbly salivation and tonic convulsions (Day 149 of dosing) and the animal died on Day 191.

Body weights: The mean body weights of the control males and females before the initiation of dosing were 9.98±0.33 and 9.48±0.45 kg respectively and the mean body weights at the end of the dosing period were 11.8±1.11 and 12.28±1.64 kg respectively. Treatment with RU-0211 was not associated with any significant changes in the body weights of the male and female animals.

Food and water consumption: The mean food consumptions of the control male and female animals were 300±0 and 288.5±23.0 g/day respectively, and the mean water consumptions were 854.5±95.7 and 665.0±98.1 ml/day respectively. There were no treatment-related changes in the food and water consumptions of the male and female animals receiving RU-0211 for 39 weeks.

Ophthalmoscopy: No ophthalmologic abnormalities were observed in the male and female dogs receiving oral doses of RU-0211.

Electrocardiography: Treatment with RU-0211 was not associated with any changes in the EKG parameters (heart rate, QRS duration, Q-T interval, P-R interval and R-waves) in the male and female animals.

Hematology: The hematological parameters of the control male and female animals are shown in the table below.

WBC $x10^3/mm^3$	RBC $x10^6/mm^3$	Hemoglo-bin (g/dl)	Hematocrit (%)	MCV (μm^3)	MCHC (g/dl)	Platelets $x10^6/mm^3$	Fibrinogen (mg/dl)	Reticulo-cytes (%)	PT (sec)	APTT (sec)
Males										
95.0±25.0	645.0±17.0	14.7±0.5	43.3±1.3	67.2±0.7	34.0±0.20	25.8±6.1	176.0±29.0	7.0±3.0	8.0±0.4	14.1±0.8
Females										
110±35.0	714.0±14.0	17.6±1.4	48.1±1.8	67.6±3.3	34.3±0.5	29.1±7.9	211±18	4.0±1.0	8.1±0.4	14.5±0.4

There were no changes in the hematological parameters in the male and female animals of any groups.

Clinical chemistry: There were no treatment-related changes in the clinical chemistry parameters in any groups of male and female animals receiving RU-0211.

Urinalysis: Urinary sodium concentrations of the high dose males were lower than the control animals in weeks 12, 25 and 38 (control levels in weeks 12, 25 and 38 were 75.2±32.9, 91.0±25.0 and 82.7±20.7 meq/L; there were 63.1%, 75.8% and 64.3% decreases respectively during these periods). In the high dose females, the urinary concentrations of sodium (71.4% and 63.2% decreases in weeks 12 and 25 respectively) and chloride (30.4% and 32.2% decreases in weeks 12 and 25 respectively) were lower than the control values in weeks 12 and 25.

Gross pathology: No gross pathological changes were observed in any groups of animals.

Organ Weights: No treatment-related changes in the organ weights were observed in the male and female animals receiving oral RU-0211.

Histopathology: Among high dose males, 1 (of 4) animal had focal hepatocytic necrosis, 2animals had atrophy of the seminiferous tubule, 1 animal had cellular infiltration of the testis, 1 animal had panarteritis of epididymides and 1 animal had cellular infiltration of the prostrate. Among females, one animal each in the mid and high dose groups had pyelitis in the right kidney and 1 control and 2 low dose animals had lymphocytic thyroiditis. The histopathological changes observed in the control male and female animals and those receiving RU-0211 are shown in the Table below.

Histopathological changes in the male and female dogs receiving oral doses of RU-0211 for 39 weeks.

Findings	Control	0.002 mg/kg	0.01 mg/kg	0.05 mg/kg
Males				
Liver				
Necrosis of hepatocyte, focal	**0/3**	**0/4**	**0/4**	**1/4**
Kidney				
Pyelitis, left	**0/3**	**0/3**	**0/3**	**1/4**
Testis				
Atrophy of seminiferous tubule	**0/3**	**0/4**	**0/4**	**2/4**
Cellular infiltration of lymphoid/plasma cells, bilateral	**0/3**	**0/4**	**0/4**	**1/4**
Epididymis				
Panarteritis, left	**0/3**	**0/4**	**0/4**	**1/4**
Prostate				
Cellular infiltration	**0/3**	**0/4**	**0/4**	**1/4**
Females				
Kidney				
Pyelitis, right	**0/4**	**0/4**	**1/4**	**1/4**
Thyroid				
Lymphocytic thyroiditis	**1/4**	**2/4**	**0/4**	**0/4**

Toxicokinetics: The plasma concentrations of RU-0211 increased with increasing doses. No apparent differences in the plasma drug concentrations were observed at 15 min and 30 min after the dosing, suggesting that the T_{max} was in between 15 and 30 minutes. The plasma drug concentrations of the male and female animals after different doses of RU-0211 are summarized in the Table below.

Plasma concentrations of RU-0211 in male and female dogs after oral administration of the drug

Males	**Day 1**		**Week 14**		**Week 27**		**Week 39**	
	15 min.	**30 min.**	**15 min.**	**30 min.**	**15 min.**	**30 min.**	**15 min.**	**30 min.**
0.002 mg/kg	29.8±41.4	39.4±12.5	61.1±52.5	23.0±16.5	56.1±39.4	39.5±10.0	29.1±40.3	29.8±6.56
0.01 mg/kg	171±106	197±173	235±194	99.5±43.0	221±123	212±86.1	206±57.6	126±25.1
0.05 mg/kg	304±259	401±256	443±719	647±330	555±494	577±541	528±643	707±334
Females								
0.002 mg/kg	7.03±14.1	46.8±29.9	67.3±23.0	21.4±15.5	27.6±18.6	45.3±75.9	33.2±42.3	10.9±12.6
0.01 mg/kg	256±163	173±88.1	172±173	221±154	368±276	212±121	103±155	96.9±73.2
0.05 mg/kg	993±499	436±217	1560±594	729±201	1190±701	585±154	821±758	847±641

In summary, in the 39-week toxicity study in Beagle dogs, male and female animals received oral doses of 0, 0.002, 0.01 and 0.05 mg/kg of RU-0211. Dose-related increases in vomiting of bubbly gastric juice, diarrhea and loose stool or watery stool were observed in both males and females at 0.01 and 0.05 mg/kg/day doses. The high dose males had decreased urinary excretion of sodium and the high dose females had decreased urinary excretion of sodium and chloride. Males receiving the high dose had focal hepatocytic necrosis (1 of 4), atrophy of the seminiferous tubule (2 of 4) and cellular infiltration of lymphoid/plasma cells in bilateral testis (1 of 4), and pyelitis in the kidney was observed in both males and females receiving the high dose (1 of 4 each). The no effect dose was 0.01 mg/kg/day and the target organs of toxicity were the testis in the males and kidney in both males and females.

2.6.6.1 Overall toxicology summary

Single and repeat dose toxicology studies with RU-0211 were conducted in rats, mice and dogs. In the acute toxicity study in rats, the minimal lethal dose was 60 mg/kg in males and 30 mg/kg in females. The clinical signs observed in rats included decreased locomotor activity, lacrimation, loose stool and dyspnea. In dogs, single oral doses up to 40 mg/kg was non-lethal, and decreased locomotor activity, loose stool/diarrhea, vomiting, lacrimation, salivation and pale buccal mucosa were observed in males and females.

In repeat dose oral toxicity study in rats and mice and dogs, loose stool or diarrhea was observed in all species, which is related to the pharmacological actions of the drug. Proliferation of the epithelial basal cells in the limiting ridge of the stomach was observed in rats and mice. In a 2-week oral toxicity study in rats, fibrous osteodystrophy of the femur and sternum and decreased cellularity of the bone marrow were observed in both males and females at a dose of 5 mg/kg. However, no effect on the bone or bone marrow was observed in the 4-week and 6-month toxicity studies in rats. Hyperplasia of the zona glomerulosa of the adrenal gland was observed in rats, mice and dogs. In the chronic 39-week oral toxicity study in dogs, atrophy of the seminiferous tubule was observed in males and pyelitis in the kidneys was observed in males and females at a dose of o.05 mg/kg/day. However, in reproductive toxicology studies, RU-0211 had no effects on the reproductive function of male rats.

2.6.6.4 Genetic toxicology

Descriptive Title: Bacterial Reverse Mutation Assay with RU-0211 (Ames test)

Sponsor's ID # Study No. 900915

Conducting Laboratory:

Date of Study Initiation/completion: February 15, 1996/ March 21, 1997

GLP Compliance: Yes

Drug Lot Number: RU-0211, Lot No. 4

Study Endpoint: Mutagenesis

METHODOLOGY:

Strains/Species/Cell line: *Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537, and *Escherichia coli* strain WP2uvrA

Dose Selection Criteria: The concentrations were selected on the basis of a concentration-range finding study in which 8 concentrations of RU-0211 (range, 1 to 5000 µg/plate) were used for each strain. There were no precipitation of the compound either in the presence or absence of S9 mix; however, inhibition of growth of all tester strains was observed at 5000 µg/plate. Based on the findings, the sponsor selected the concentration range of 78.1-5000 µg/plate with a dilution ratio of 2 (78.1, 156.3, 321.5, 625, 1250, 2500 and 5000 µg/plate) for this study.

Test Agent Stability Considerations: The stability of the test substance was determined by measuring the concentrations of the samples. The concentrations ranged from — of the specified concentrations.

Metabolic Activation System: Rat liver microsomal S9 mix — Lot No. 95121510) was used as the metabolic activator.

Controls: Dimethyl sulfoxide (DMSO) was used as the negative control. As positive controls, AF-2 (2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide), sodium azide, 9AA (9-Aminoacridine hydrochloride), and 2AA (2-Aminoanthracene) were used.

Exposure conditions: The tests were performed by the preincubation method with or without S9 mix. The culture medium containing the bacterial suspensions (with the test substance or controls) were shake-

cultured for 20 minutes at 37^{0}C and top agar was added to the mixture. The mixture was poured over minimum glucose agar plates and the plates were incubated at 37^{0}C for 48 hours.

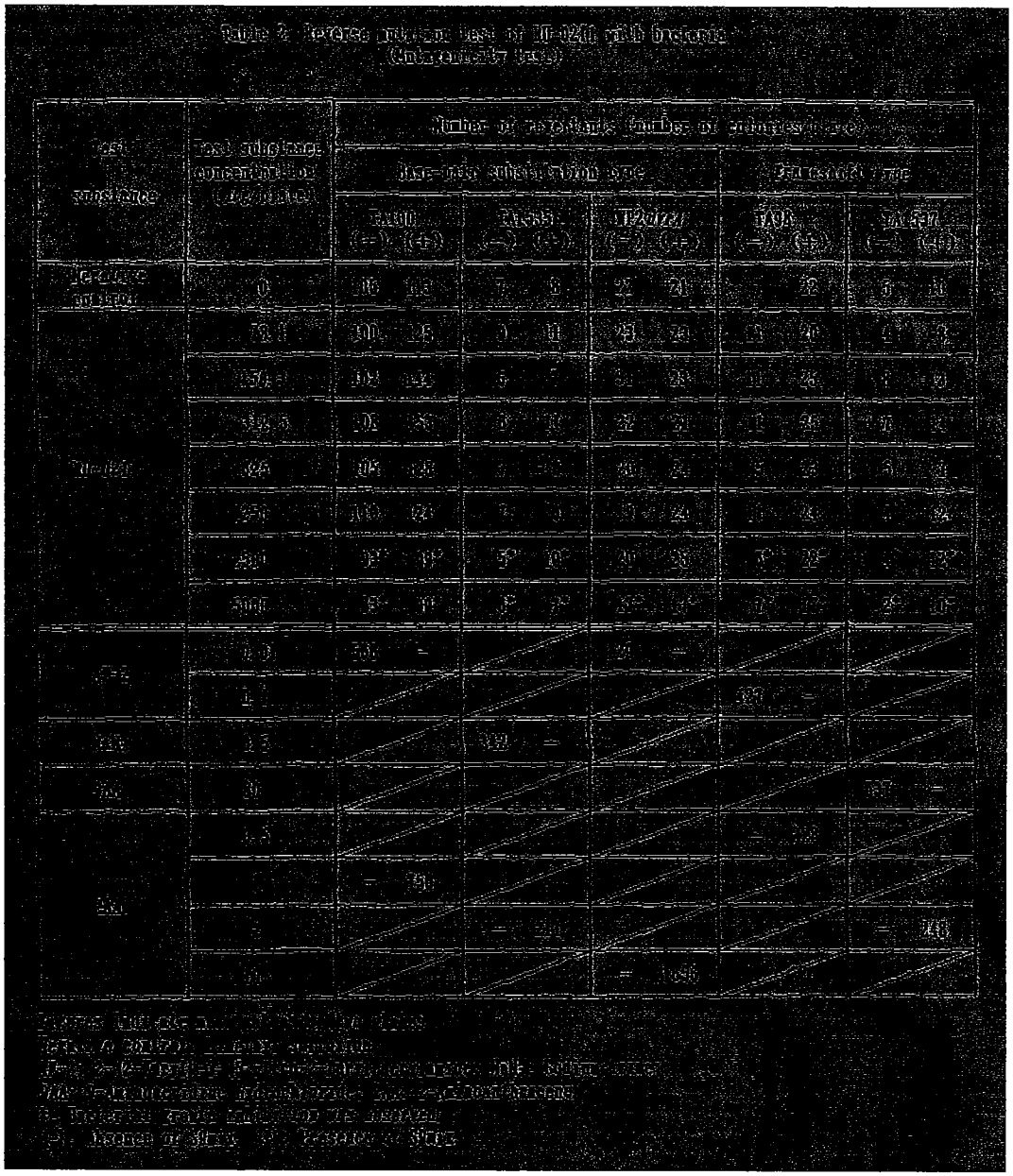
Analysis: The numbers of revertant colonies were visually counted. The mean of the number of revertant colonies at each concentration was determined and compared with the positive controls.

Criteria for Positive Results: When the number of revertant colonies in any groups was more than two times the negative control and there was a dose dependent increase in the number of revertant colonies, the test result was considered positive.

RESULTS

Study Validity: In aseptic tests, no contamination was found in the test substance or S9 mix. In addition, the numbers of revertant colonies in the positive and negative controls were within the historical background data of the laboratory.

Study Outcome: The numbers of revertant colonies in the RU-0211 groups were less than two-fold of that in the negative control with or without S9 mix. The numbers of revertant colonies for the positive controls were clearly increased for each tester strain. RU-0211 caused significant inhibition of growth of all strains at 5000 μg/plate. A concentration of 2500 μg/plate also caused significant inhibition of growth of all strains except *E. coli* WP2uvrA. The numerical data is shown in the sponsor-provided table below.



SUMMARY:

The results indicated that RU-0211 did not cause any significant increase in the number of revertant colonies in any of the tester strains examined. Thus, the compound was not mutagenic in the test systems under the experimental conditions.

Chromosomal Aberration Test in Cultured Mammalian Cells.

Study No. 971415

Descriptive Title: In vitro chromosomal aberration test with RU-0211 in Chinese hamster lung (CHL) cells.

Conducting Laboratory:

Date of Study Initiation/completion: February 15, 1996/ March 21, 1997

GLP Compliance: Yes

Drug Lot Number: RU-0211, Lot No. 4 and 5

Study Endpoint: Clastogenicity

METHODOLOGY:

Strains/Species/Cell line: Chinese hamster lung (CHL/IU) fibroblast cell line.

Dose Selection Criteria: The concentrations were selected on the basis of a cell growth inhibition study in which the cells were grown for 48 hours with 10 concentrations (4.883 to 2500 μg/ml, with a dilution factor of 2) of RU-0211 with or without metabolic activation. There were decreased viability of the cells at 78.13 μg/ml and higher concentrations without metabolic activation. In the presence of the metabolic activation, cell viability was decreased at 312.5 μg/ml and higher concentrations. The concentrations of RU-0211 that caused 50% inhibition of the cell growth (IC_{50}) without and with metabolic activation were 95.8 μg/ml and 573.6 μg/ml respectively. The highest concentrations used in the chromosomal aberration test were higher than the IC_{50} values both in the presence (75, 150, 300, 600 and 1200 μg/ml) and absence (12.5, 25, 50, 100 and 200 μg/ml) of metabolic activation.

Test Agent Stability Considerations: The concentrations of RU-0211 in the samples for both the direct and metabolic activation methods were determined. The concentrations for the direct method ranged from — of the expected concentrations. In the samples for the metabolic activation method, the concentrations ranged from —

Metabolic Activation System: Rat liver microsomal S9 mix (— Lot No. 95121510) was used as the metabolic activator.

Controls: Dimethyl sulfoxide (DMSO) was used as the negative control. As positive controls, mitomycin C (MMC; 0.1 μg/ml) and dimethylnitrosamine (DMN; 1000 μg/ml) were used.

Exposure conditions: The cells were incubated with the test substance or controls for 24 or 48 hours with or without S9 mix. In both the direct and metabolic activation methods, 0.1 ml of Colcemid solution (10 µg/ml) was added to obtain metaphase cells. The chromosome specimens were prepared from each sample and examined.

Analysis: One hundred metaphase cells per plate and 200 metaphase cells per concentration were observed in areas where the chromosomes were well spread. For numerical aberration, only polyploidy was observed. Structural aberrations were classified into (a) chromatid gaps (ctg), (b) chromosome gaps (csg), (c) chromatid breaks (ctb), (d) chromosome breaks (csb), (e) chromatid exchanges (cte), (f) chromosome exchanges (cse) and (g) fragmentation (frg).

Criteria for Positive Results: The results were considered negative when the number of cells with numerical or structural aberrations was less than 5%. When the number of aberrations was between 5% and 10%, the results were judged 'pseudo-positive'. The results were considered positive when the number of aberrations were more than 10% and increased with increasing concentrations of the test agent. The criteria for positive results, used by the sponsor, are not universally accepted.

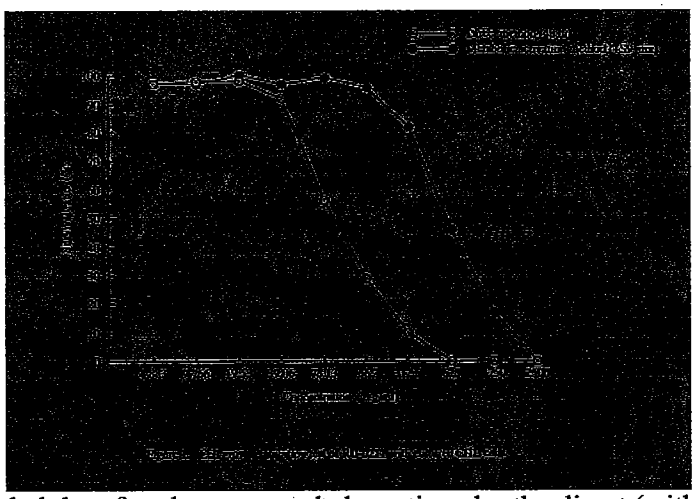
RESULTS

Study Validity: The tests were considered valid when the number of cells with chromosomal aberration was less than 5% in the negative control groups, when the number of cells with chromosomal aberration other than gaps were more than 10%, the findings were within the background data of the testing facility and there was no factor affecting the study systems. Considering the above criteria, the tests were valid both in the direct and metabolic activation methods. However, these criteria used by the sponsor, are not the acceptable criteria for study validity.

Study Outcome: In the direct method (without metabolic activation), cell viability was decreased at 78.13 µg/ml or higher concentrations. The frequencies of cells with structural aberrations of chromosomes (ctg, ctb, cte and frg) were increased at 200 µg/ml, being 27.5% and 61.4% for 24 hours and 48 hours treatments respectively. There was inhibition of cell growth at this concentration and only 44 cells were observed in 2 plates.

In the presence of metabolic activation, cell viability was decreased at 312.5 µg/ml or higher concentrations. The frequency of cells with structural chromosome aberrations was considered 'pseudo-positive', being 8% at 1200 µg/ml. The types of aberrations were in the chroma tid that included ctb and cte.

The inhibition of cell growth by RU-0211 by the direct and metabolic activation method is shown in the figure below.



The sponsor-provided data for chromosomal aberrations by the direct (without metabolic activation) and metabolic activation methods are summarized in the tables below.

Chromosomal aberrations in CHL cells by the direct method.

Test Substance	Conc. (μg/ml)	Treatment Time (Hr)	No. of Metaphase examined	Incidence of poly-ploidy(%)	Judge-ment	No. of cells with Chromosome aberrati		Incidence		Judgement
						(+g)	(-g)	(+g)	(-g)	
DMSO	-	24	200	0	-	0	0	0	0	-
RU-0211	12.5	24	200	0	-	0	0	0	0	-
	25	24	200	1.0	-	1	0	0.5	0	-
	50	24	200	1.0	-	1	0	0.5	0	-
	100	24	200	1.5	-	4	4	2.0	2.0	-
	200	24	200	0	-	55	52	27.5	26.0	+
Mitomycin C	0.1	24	200	0	-	123	123	61.5	61.5	+
DMSO	-	48	200	0.5	-		1	1	0.5	-
RU-0211	12.5	48	200	1.0	-		0	0	0	-
	25.0	48	200	1.0	-		2	2	1.0	-
	50	48	200	0.5	-		1	1	0.5	-
	100	48	200	0	-		6	4	2.0	-
	200	48	44	0	-		27	27	61.4	+
Mitomycin	0.1	48	200	0	-		188	188	94.0	+

C										

-, negative (less than 5%); +, positive (≥10%); (+g), total no. of aberrant cells including gap; (-g), total no. of aberrant cells excluding the gap.

Chromosomal aberrations in CHL cells by the metabolic activation method.

Test Substance	Conc. (μg/ml)	No. of Metaphase examined	Incidence of poly-ploidy(%)	Judge-ment	No. of cells with Chromosome aberrati		Incidence (%)		Judgement
					(+g)	(-g)	(+g)	(-g)	
DMSO	-	200	1.0	-	3	3	1.5	1.5	-
RU-0211	75	200	0	-	0	0	0	0	-
	150	200	0.5	-	1	1	0.5	0.5	-
	300	200	0.5	-	1	1	0.5	0.5	-
	600	200	0	-	4	4	2.0	2.0	-
	1200	200	0.5	-	16	16	8.0	8.0	±
Dimethyl-Nitrosamine	1000	200	0	-	171	171	85.5	85.5	+

-, negative (less than 5%); ±, 'suspicious'; +, positive (≥10%); (+g), total no. of aberrant cells including gap; (-g), total no. of aberrant cells excluding the gap.

An additional test was conducted with only the higher concentrations of RU-0211 (100, 150 and 200 μg/ml by the direct method (without metabolic activation) and 600, 900 and 1200 μg/ml by the metabolic activation method. The frequencies of cells with structural chromosome aberrations (mainly ctb and cte) were higher at 150 μg/ml and higher concentrations (10 to 34% at 24 hr. and 26 to 49% at 48 hr. treatment periods). The viability of cells at these concentrations decreased with increasing concentrations (30 to 39% at 24 hr. and 14 to 36% at 48 hr. treatment). In the presence of metabolic activation, the numbers of cells with chromosome aberrations were less than 5%. Thus, the chromosomal aberrations with RU-0211 in the absence of metabolic activation were observed at concentrations that produced >60% inhibition of cell growth (<40% viability). The chromosomal aberrations observed with RU-0211 in the absence of metabolic activation could be related to its cytotoxicity.

The incidences of structural chromosome aberrations in cells treated with RU-0122 and the positive and negative controls by the direct incubation method are summarized in the table below.

Test Substance	Conc. (μg/ml)	Treatment Time (Hr)	No. of Metaphase examined	No. of cells with chromosome aberrations		Incidence		Judgement	Survival Ratio (%)
				(+g)	(-g)	(+g)	(-g)		
DMSO	-	24	200	2	2	1.0	1.0	-	100
RU-0211	100	24	200	4	3	2.0	1.5	-	68
	150	24	200	20	19	10.0	9.5	+	39
	200	24	200	68	68	34.0	34.0	+	30
Mitomycin C	0.1	24	200	104	103	52.0	51.5	+	77
DMSO	-	48	200	2	2	1.0	1.0	-	100
RU-0211	100	48	200	4	4	2.0	2.0	-	65
	150	48	200	52	52	26.0	26.0	+	36
	200	48	53	26	26	49.0	49.0	+	14
Mitomycin C	0.1	48	200	184	184	92.0	92.0	+	67

-, negative (less than 5%); +, positive (≥10%); (+g), total no. of aberrant cells including gap; (-g), total no. of aberrant cells excluding the gap.

The incidences of structural chromosome aberrations in cells treated with RU-0122 and the positive and negative controls in the presence of metabolic activation are summarized in the table below.

Test Substance	Conc. (μg/ml)	No. of Metaphase examined	No. of cells with chromosome aberrations		Incidence		Judgement	Survival Ratio (%)
			(+g)	(-g)	(+g)	(-g)		
DMSO	-	200	0	2	0	0	-	100
RU-0211	600	200	1	3	0.5	0	-	69
	900	200	7	19	3.5	3.5	-	54
	1200	200	9	68	4.5	4.0	-	38
D-methyl-Nitrosamine	1000	200	141	103	70.5	70.5	+	78

-, negative (less than 5%); +, positive (≥10%); (+g), total no. of aberrant cells including gap; (-g), total no. of aberrant cells excluding the gap.

SUMMARY:

In vitro chromosomal aberration with RU-0211 was investigated in cultured CHL cells in the absence and presence of metabolic activation. The frequencies of cells with structural aberrations of chromosomes were increased with RU-0211 in the absence of metabolic activation. However, chromosomal aberrations were observed at concentrations (≥150 μg/ml) of RU-0211 that were cytotoxic to the cells (>60% cytotoxicity). Thus, it is possible that cytotoxicity of the compound contributed to the

chromosomal aberrations observed in the absence of metabolic activation. RU-0211 is a prostaglandin analog and the prostaglandins, in general, do not possess any structural alert for genotoxic potential.

In vitro Mouse Lymphoma Cell (L5178Y) Forward Gene ($TK^{+/-}$) Mutation Assay

(Study Report # RTU/SR01-031; Cross Reference #142-106)

Testing Laboratory:

Study Start and Completion Dates: October 16, 2000 and February 19, 2001

GLP and QAU Compliance Statement: The sponsor submitted a statement of compliance with GLP regulations and QAU.

Methods:

The mouse lymphoma L5178Y cell line, heterozygous at the TK locus, was used in the study. The cells were cultured in RPMI 1640 medium supplemented with horse serum (10%), Pluronic F-68, L-glutamine, sodium pyruvate, penicillin and streptomycin. The treatment medium was Fischer's medium with the same supplements with 5% horse serum. Dimethyl sulfoxide (DMSO) was used as the vehicle control (1%) and methyl methanesulfonate (MMS) methylcholanthrene (MCA) were used as positive controls in the absence and presence of metabolic activation respectively. Male rat (treated with Aroclor 1254) liver S9 fraction (supplemented with NADP and isocitrate) was used as the metabolic activator. Preliminary dose range finding studies were conducted in the presence (approximately 4 hours incubation) and absence (approximately 4 hours and 24 hours incubation) of metabolic activation. The initial assays were conducted with a four-hour treatment period both in the absence and presence of metabolic activation. The repeat assays were conducted with a 24-hour incubation period in the absence and a 4-hour incubation period in the presence of metabolic activation. The mutant frequency was calculated as the ratio of the total number of mutant colonies to the total number of cells seeded, adjusted by the cloning efficiency. The cytotoxicity of each treatment, measured as relative total growth (RTG), was obtained from the relative suspension growth of the cells multiplied by the relative cloning efficiency at the time of selection. Both the small and large colonies were quantified for all cultures.

The assay was acceptable when (a) The average absolute cloning efficiency of the vehicle controls were between 60% and 130%, (b) the average suspension growth of the vehicle controls for 2 days was an 8-fold increase over the original cell concentrations, (c) at least one of the positive control cultures in each trial induced a mutant frequency of at least $200x10^{-6}$. The test article was considered positive when there were dose-dependent increases of 2-fold or greater in mutant frequency over the current background mutant frequency.

Results:

In the preliminary dose range-finding study, the concentrations of RU-0211 ranged from 19.7 to 2500 μg/ml. With the 4-hour treatment period, RU-0211 had similar cytotoxicity both in the absence and presence of metabolic activation. The compound was highly cytotoxic at concentrations of 1250 to 2500 μg/ml (less than 10% relative suspension growth). In the absence of metabolic activation with a 24-hour treatment period, RU-0211 was highly cytotoxic at concentrations above 78.5 μg/ml.

In the initial assay in the absence of metabolic activation, 100, 200, 400, 600, 650, 700, 750, 800, 900, 950, 1000, 1200 and 1400 μg/ml concentrations were used. The sponsor stated that the 1000 μg/ml and higher concentrations were excessively cytotoxic and were terminated. At 950 μg/ml, there were more than 2-fold increases in the mutant frequency as compared with the concurrent vehicle control. The sponsor repeated the assay with a 24-hour treatment period in the absence of metabolic activation and in the repeat assay, 12.5, 25.0, 50.0, 75.0, 100, 125, 150, 200, 250, 300 and 400 μg/ml concentrations were used. Treatments at and above 125 μg/ml were terminated because of excessive cytotoxicity. In the repeat assay, the 100 μg/ml concentration produced a nearly 2-fold increase in the mutant frequency for small colonies. The mutant frequencies of the small and large colonies for the initial and repeat assays are shown in the Table below.

Table: Mouse lymphoma cell (L5178Y) forward gene ($TK^{+/-}$) mutation assay without metabolic activation.

Initial Assay (4-hour treatment):

Treatment	Cloning Efficiency		Relative Growth	Mutant Frequency (10^{-6})		
	Absolute %	Relative %	(%)	Total	Small	Large
Vehicle Control (DMSO)	105.8	109.0	109.1	46.8	21.9	24.8
	88.4	91.0	91.8	52.3 (46.8)	23.9 (21.7)	28.4 (25.1)
	97.1	100.0	99.0	41.2	19.2	22.1
MMS (13 μg/ml)	47.1	48.5	24.0	235.5 *	158.3 *	77.2 *
	42.8	44.1	25.8	380.5 *	242.0 *	138.4 *
RU-0211 (μg/ml)						
400	82.6	85.0	68.6	48.5	26.0	22.5
600	105.3	108.4	45.4	45.9	29.4	16.6
650	108.2	111.4	41.1	52.8	24.9	27.9
700	90.2	92.9	36.5	54.4	33.9	20.6
750	89.8	92.5	34.9	42.5	25.1	17.4
800	99.5	102.4	21.4	43.9	20.5	23.4
900	75.5	77.7	16.2	47.2	24.6	22.7
950	77.6	80.0	12.4	95.6 *	55.3 *	40.3
Repeat Assay (24-hour treatment)						
Vehicle Control (DMSO)	83.3	99.8	100.4	69.9	31.4	38.4
	87.6	105.1	111.5	51.0 (62.8)	24.5 (28.5)	26.6 (34.2)
	79.3	95.1	88.7	67.4	29.8	37.6
MMS (6.5 μg/ml)	44.5	53.4	8.5	650.6	444.9	205.7
	50.4	60.4	6.7	369.0	226.0	143.0
RU-0211 (μg/ml)						
12.5	79.5	95.3	64.5	66.4	25.2	41.2
25.0	82.7	99.2	88.0	67.3	29.5	37.8
50.0	96.6	115.8	63.2	66.3	31.6	34.7
75.0	80.2	96.2	38.5	82.1	40.4	41.7
100	71.2	85.4	19.8	110.3	56.2	54.2

***, more than 2-fold increase in the mutant frequency; the values in the parenthesis is the mean of 3 values.**

In the initial assay in the presence of metabolic activation, 200, 300, 400, 500, 600, 650, 700, 750, 800, 900, 950, 1000 and 1200 μg/ml concentrations were used. The sponsor

stated that the treatments at 700 µg/ml and higher concentrations were terminated because of excessive cytotoxicity. RU-0211, at concentrations up to 650 µg/ml, did not produce a 2-fold increase in the mutant frequency in the presence of metabolic activation. In the repeat assay, there were no increases in the mutant frequency at concentrations up to 700 µg/ml. The mutant frequencies for the small and large colonies in the presence of metabolic activation are shown in the Table below.

Table: Mouse lymphoma cell (L5178Y) forward gene ($TK^{+/-}$) mutation assay with metabolic activation.

Initial Assay (4-hour treatment):

Treatment	Cloning Efficiency		Relative Growth	Mutant Frequency (10^{-6})		
	Absolute %	Relative %	(%)	Total	Small	Large
Vehicle Control (DMSO)	82.4	103.0	96.8	61.4	26.0	35.3
	75.8	94.8	102.0	67.6 (66.9)	29.7 (30.4)	37.9 (36.4)
	81.8	102.3	100.6	71.6	35.6	36.0
MCA (µg/ml) 2	66.0	83.2	48.5	330.6 *	173.8 *	156.8 *
4	66.0	82.5	48.1	298.6 *	151.0 *	147.7 *
RU-0211 (µg/ml)						
200	87.1	108.9	98.6	67.2	39.7	27.6
300	96.2	120.2	88.9	58.2	27.2	31.0
450	90.9	113.6	62.0	72.4	26.4	46.0
500	104.6	130.7	56.3	55.7	24.7	31.0
600	109.1	136.4	22.3	73.0	43.3	29.7
650	83.3	104.1	20.3	84.3	53.3	31.0
Repeat Assay (4-hour treatment)						
Vehicle Control (DMSO)	116.6	124.2	107.8	39.0	13.7	25.3
	72.0	76.7	87.6	58.1 (50.1)	21.7 (17.9)	36.4 (32.2)
	92.9	99.0	98.1	53.2	18.4	34.8
MCA (µg/ml) 2	68.6	73.1	49.0	229.7 *	105.0 *	124.7 *
4	63.1	67.2	35.8	251.3 *	102.0 *	149.3 *
RU-0211 (µg/ml)						
200	78.0	83.2	77.4	66.7	28.9	37.8
300	77.5	82.6	58.0	67.6	22.5	45.1
400	66.4	70.7	54.4	71.8	30.7	41.1
500	99.6	106.2	37.1	60.6	23.4	37.2
550	86.9	92.6	35.2	57.3	23.0	34.3
600	88.9	94.8	26.2	58.9	29.0	29.9
650	78.0	83.1	16.3	71.8	28.9	42.2
700	112.7	120.2	20.4	37.7	16.1	21.6

*, more than 2-fold increase in the mutant frequency; the values in the parenthesis is the mean of 3 values.

The *in vitro* mouse lymphoma cell (L5178Y) forward gene ($TK^{+/-}$) mutation assay with RU-0211 was conducted in the presence or absence of metabolic activation with S9 mix. In the initial assay, with 4-hour incubation, RU-0211 (950 µg/ml) caused an increase in the mutant frequency (small colonies) in the absence of metabolic activation. The sponsor repeated the assay with a 24-hour incubation period and in the repeat assay, RU-0211 caused nearly 2-fold increases in the mutant frequency for the small colonies at an RTG of 19.8%. In the presence of metabolic activation, there were no increases in the mutant frequency either in the initial assay or the repeat assay.

Bone Marrow Micronucleus Test with RU-0211 in Mice by the Oral Route.

Sponsor's ID # Study No. 940315

Descriptive Title: In vivo bone marrow micronucleus test in mice by the oral route.

Conducting Laboratory:

Date of Study Initiation/completion: February 01, 1996/ March 21, 1997

GLP Compliance: Yes

Drug Lot Number: RU-0211, Lot No. 4

Study Endpoint: Frequency of micronucleated immature (polychromatic) erythrocytes.

METHODOLOGY:

Strains/Species/Cell line: CD-1 (ICR) male mice (26.1g-30.8 g, 6 weeks old).

Dose Selection Criteria: The concentrations were selected on the basis of lethality tests with RU-0211 in mice. In the first study, the animals received oral doses of RU-0211 at 62.5, 125, 250 and 500 mg/kg (given 2 times at 24 hr intervals). Abnormal clinical signs and deaths were observed at all doses. In the second study, RU-0211 doses of 3.8, 7.5, 15, 30 and 60 mg/kg were used. There were deaths at 15 (1/3), 30 (3/3) and 60 (2/3) mg/kg doses and loose stool and diarrhea were observed at all doses. At 3.8 and 7.5 mg/kg doses, decreased locomotor activity, loose stool and diarrhea were observed and there were no deaths. So, the sponsor selected 8 mg/kg (between 7.5 and 15) as the high dose and 2 and 4 mg/kg as the low and medium doses for the micronucleus test.

Test Agent Stability Considerations: The concentrations of RU-0211 in the samples used for both lethality testing and micronucleus assay were determined before use. The concentrations of the compound in the samples ranged from 95.3% to 104.3% of the specified concentrations.

Metabolic Activation System: N/A.

Controls: Polyethylene glycol 400 (PEG-400) was used as a negative control. As the positive control, mitomycin C (MMC) was used.

Exposure conditions: The animals (5/group) received oral doses of RU-0211 at 2, 4 and 8 mg/kg; two doses were administered at 24-hour intervals. The vehicle (negative control) was also administered at the same intervals. The positive control (MMC) was administered once by SC injections. The bone marrow cells from RU-0211 and negative control treated animals were harvested at 24 and 48 hours after the second dose; for positive control treated animals, the sampling was done 24 hours after the dosing.

Analysis: The number of micronucleated erythrocytes, polychromatic erythrocytes (PCE) and polychromatic and normochromatic erythrocytes (NCE) were counted. The frequency of PCE with micronucleus (MNPCE) and the frequency of PCE compared to total erythrocytes (PCE/(PCE+NCE)) were calculated. Statistical significance was determined between the negative control group and R-0211 groups as well as between negative and positive control groups (Student's t-test).

Criteria for Positive Results: The results were considered positive when there were significant and dose-dependent increases in the MNPCE in the treatment groups as compared to the negative control.

RESULTS

Study Validity: The test was considered valid when the frequencies of micronucleated cells in the negative and positive control groups fell within the established acceptable ranges based on the background data obtained in the testing facility.

Study Outcome: There was no significant increase in the frequency of MNPCE in any treatment groups as compared with the negative control group. There was also no significant increase in the PCE/(PCE+NCE) in any treatment groups. In the positive control group, the frequency of MNPCE was significantly higher as compared to the negative control group. The frequencies of MNPCE and PCE/(PCE+NCE) in the control and treatment groups are summarized in the table below.

Group	Sampling time (hr)	Number of animals	MNPCE (%)		PCE/(PCE+NCE) (%)	
			Mean±S.D.	Min./Max	Mean±S.D.	Min./Max
PEG-400	24	5	0.14±0.114	0/.03	48.4±6.43	37.6/54.8
	48	5	0.10±0.071	0/0.2	53.1±4.43	47.5/58.1
RU-0211	24	5	0.14±0.114	0/0.3	48.9±10.54	34.5/57.9
(2 mg/kg)	48	5	0.08±0.084	0/0.2	51.7±4.78	46.6/58.3
RU-0211	24	5	0.20±0.122	0.1/0.4	50.3±3.16	47.9/55.2
(4 mg/kg)	48	5	0.14±0.114	0/0.3	54.2±5.99	48.4/62.2
RU-0211	24	5	0.20±0.122	0.1/0.4	39.5±15.23	21.6/59.3
(8 mg/kg)	48	4	0.13±0.050	0.1/0.2	49.3±1.55	47.1/50.8
Mitomycin C	24	5	7.12±1.588	5.2/9.1	44.9±8.89	33.6/56.7
(2 mg/kg)						

Summary:

The potential for RU-0211 to cause chromosomal aberration was assessed in bone marrow micronucleus test in mice. The frequency of micronucleated cells did not increase in the RU-0211 treated groups at doses up to 8 mg/kg. Thus, RU-0211 was not clastogenic in mice under the experimental conditions.

2.6.6.5 Carcinogenicity

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

P/T REVIEWER(s): Sushanta Chakder, Ph.D.
DATE:
IND/NDA: NDA 21-908
DRUG CODE#: RU-0211
CAS#: 209859-87-0
DIVISION(s): Division of Gastroenterology Products, HFD-180
DRUG NAME(s): Lubiprostone
SPONSOR: Sucampo Pharmaceuticals, Inc., Bethesda, MD.
LABORATORY: —
CARCINOGENICITY STUDY REPORT DATE: February 11, 2005
THERAPEUTIC CATEGORY: Laxative.
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Prostaglandin Analog.
MUTAGENIC/GENOTOXIC: RU-0211 was not genotoxic in a battery of genotoxicity assays, including the Ames test, the *in vitro* chromosome aberration assay in Chinese hamster lung (CHL) cells, the *in vitro* mouse lymphoma cell forward gene mutation assay and the *in vivo* mouse bone marrow micronucleus assay.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.):

MOUSE STUDY DURATION (weeks): 104 Weeks
STUDY STARTING DATE: February 19, 2002
STUDY ENDING DATE: February 11, 2005
MOUSE STRAIN: Crl:B6C3F1 mice
ROUTE: Oral gavage

DOSING COMMENTS: The doses for the 2-year mouse carcinogenicity study (25, 75, 200 and 500 µg/kg/day) was based on toxicity endpoints and the MTD from a 13-week oral toxicity study in mice. In the 13-week oral gavage toxicity study in mice, the MTD was between 100 and 1000 µg/kg/day. Diarrhea and histopathological changes in the stomach (edema, acanthosis and hyperkeratosis) were observed in males and females at 1000 and 5000 µg/kg/day doses. Based on these findings, 500 µg/kg/day was selected as the high dose for the two year mouse carcinogenicity study. The doses for the mouse carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001).

NUMBER OF MICE:
- Control- (C1): 55 animals/sex
- Low Dose (LD): 55 animals/sex
- Low Middle Dose (LMD): 55 animals/sex
- High Middle Dose (HMD): 55 animals/sex
- High Dose (HD): 55 animals/sex

MOUSE DOSE LEVELS* (mg/kg/day):
- Low Dose: 25 µg/kg/day
- Low Middle Dose: 75 µg/kg/day
- High Middle Dose: 200 µg/kg/day
- High Dose: 500 µg/kg/day

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): The dose selection for the 2-year mouse carcinogenicity study was based on the toxicity endpoints and the MTD from a 13-week oral toxicity study in mice. In the 13-week oral gavage toxicity study in mice, the MTD was between 100 and 1000 µg/kg/day. Diarrhea and histopathological changes in the stomach (edema, acanthosis and hyperkeratosis) were observed in males and females at 1000 and 5000 µg/kg/day doses. Based on these findings, doses of 25, 75, 200 and 500 µg/kg/day were selected for both males and females for the 2-year carcinogenicity study.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): The doses for the 2-year carcinogenicity study in mice were concurred by the CDER Executive CAC Committee (November 27, 2001; see Appendix 3).

MOUSE CARCINOGENICITY (conclusion: negative; positive): There was a positive trend for incidences of Harderian gland carcinoma in female mice treated with RU-0211 (p=0.0493, sponsor). A positive trend was also observed for the combined incidences of Harderian gland adenoma and carcinoma (p=0.0122, sponsor; p=0.0162, CDER Statistician) in female mice.

However, according to the CDER standard, to be statistically significant, the p values for the common tumors should be less than 0.005. Thus, according to the CDER criteria, the incidences of Harderian gland adenoma + carcinoma in female mice were not significant. The incidences of Harderian gland carcinoma at 200 (2/55; 3.6%) and 400 (2/55; 3.6%) µg/kg doses in female mice were higher than the historical control incidences from the conducting laboratory in this strain of mice (0% to 2%; mean, 0.67%). The incidences of Harderian gland carcinoma in female mice were also higher than the background incidences in this strain of mice (0% to 2%, mean 1.3%; NTP database).

MOUSE TUMOR FINDINGS: Following oral administration of RU-0211 to female mice for 104 weeks, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%); p=0.0493] and combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122].

MOUSE STUDY COMMENTS: The dose selection for the 2-year mouse carcinogenicity was appropriate, and concurred by the CDER Executive CAC Committee. The conduct of the study was appropriate and acceptable.

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE

1. **Study No.:** 7142-109
2. **Name of Laboratory:** —
3. **Strain:** Crl:B6C3F1 mice
4. **No./sex/group:** 55 animals/sex/group
5. **Doses (0, L, M, and H):** RU-0211 was administered to groups of male and female mice at oral (gavage) doses of 25, 75, 200 and 500 µg/kg/day.

Basis for dose selection stated. Yes. The dose selection for the 2-year mouse carcinogenicity study with RU-0211 was based on the toxicity endpoints, and the MTD from a 13-week oral toxicity study in mice. In the 13-week oral gavage toxicity study in mice, the MTD was between 100 and 1000 µg/kg/day. From these findings, 500 µg/kg/day was selected as the high dose for the 2-year mouse carcinogenicity study. The doses for the mouse carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001).

6. **Interim sacrifice:** No
7. **Total duration (weeks):** 104 Weeks
8. **Week/site for first tumor:**

	Male	**Female**
Control	69/Thyroid follicular adenoma.	79/Vascular hemangioma, sub-cutaneous tissue Schwannoma
Low dose	89/Bone, osteosarcoma	43/Multiple organs, hematopoietic tumor
Low mid-dose	70/Vascular neoplasia - hemangiosarcoma	72/ Multiple organs, hematopoietic tumor
High mid-dose	63/ Vascular neoplasia - hemangiosarcoma	46/ Vascular neoplasia - hemangiosarcoma
High dose	79/ Vascular neoplasia - hemangiosarcoma	74/ Multiple organs, hematopoietic tumor

9. **Number alive at termination:**

	Male	**% survival**	**Female**	**% survival**
Control	38/55	69%	40/55	73%
Low dose	42/55	76%	36/55	65%
Low middle dose	35/55	64%	43/55	78%
High middle dose	32/55	58%	32/55	58%
High dose	42/55	76%	33/55	60%

Statistical methods used: One-way analysis of variance (ANOVA) was used to analyze the effects of the drug on body weights, food consumption, and hematology parameters. If ANOVA on homogenous or transformed data was significant, Dunnett's multiple comparison t-test was used for pairwise comparisons between treated and control groups. Graphical evaluation of survival was performed using Kaplan-Meier product limit estimate curves. Statistical significance was determined using Cox-Tarone binary regression-based tests and Gehan-Breslow

nonparametric score-based tests. Nonneoplastic lesions were analyzed by the Cochran-Armitage test for trend and Fisher-Irwin exact test. Palpable tumors were analyzed by life table technique (log rank test). Occult or internal tumors were classified as fatal or incidental. Exact permutation test was then used to test tumor incidence rates. When the number of tumor occurrences was more than eight, Peto's mortality-prevalence test was used to analyze the tumor data. Rare tumors were tested at the 5%, one-tailed probability level, and common tumors at the 1%, one-tailed probability level.

10. **Attach tumor and non-tumor data for each tissue:** See Appendix 1.

Study title: **104-Week Oral Gavage Carcinogenicity Study with RU-0211 in Mice**

Key study findings: Following oral administration of RU-0211 to female mice for 104 weeks, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%); p=0.0493] and combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122, sponsor; p=0.0162, CDER Statistician)]. However, according to the CDER standard, to be statistically significant, the p values for the common tumors should be less than 0.005. Thus, according to the CDER criteria, the incidences of Harderian gland adenoma + carcinoma in female mice were not significant.
Study number: 7142-109
Volume #, and page #: The study was submitted electronically.
Conducting laboratory and location: —
Date of study initiation: February 19, 2002.
Date of study report: February 11, 2005.
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: RU-022 in median chain triglyceride (MCT); Lot nos. 020212, 021007, 021203, YY030602, YY031119; Purity —
CAC concurrence: Yes. The doses used for the carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001; See Appendix # 3).

Study Type: 2-year carcinogenicity study.
Species/strain: Crl:B6C3F1 mice
Number/sex/group; age at start of study: There were 55 animals/sex/group. The animals were approximately 51 days old; body weights: Males, 16.9 to 31.7 g, and Females, 11.9 to 23.6 g.
Animal housing: Animals were housed individually in suspended, wire-mesh, stainless-steel cages.
Formulation/vehicle: The drug was dissolved in medium chain triglyceride (MCT) and stored refrigerated at 2-8^{0}C. Control animals received the vehicle (MCT).
Drug stability/homogeneity: Samples of the dosing solutions of RU-0211 in MCT were analyzed by the sponsor, and the concentrations were within acceptable range.

Methods:

Doses: The doses of RU-0211 used in the carcinogenicity study were 25, 75, 200 and 500 µg/kg/day (1ml/kg/day).

Basis of dose selection: The dose selection for the 2-year mouse carcinogenicity study was based on the MTD from a 13-week oral toxicity study with RU-0211 in mice. In the 13-week oral gavage toxicity study in mice, diarrhea and histopathological changes in the stomach (edema, acanthosis and hyperkeratosis) were observed in males and females at 1000 and 5000 µg/kg/day doses. The MTD was between 100 and 1000 µg/kg/day. For the 2-year mouse carcinogenicity study, 500 µg/kg/day was selected as the high dose, and the low, low-mid and high-mid doses were 25, 75 and 200 µg/kg/day, respectively. The doses

for the mouse carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001; see Appendix 3).

Restriction paradigm for dietary restriction studies: None

Route of administration: The drug was administered by oral gavage.

Frequency of drug administration: The doses were administered once a day, at a dosing volume of 1 ml/kg.

Dual controls employed: No.

Interim sacrifices: No.

Satellite PK or special study group(s): For toxicokinetic analysis, satellite groups consisting of 40 mice/sex were administered 25, 75, 200 and 500 µg/kg/day doses of the drug for 26 weeks. Blood samples were collected on Day 1 and Day 26 at 0.5, 1, 2, 6 and 24 hours post-dose.

Deviations from original study protocol: There were minor deviations in the study protocol, which are not expected to affect the outcome of the carcinogenicity study.

Statistical methods: One-way analysis of variance (ANOVA) was used to analyze the effects of the drug on body weights, food consumption, and hematology parameters. If ANOVA on homogenous or transformed data was significant, Dunnett's multiple comparison t-test was used for pairwise comparisons between treated and control groups. Graphical evaluation of survival was performed using Kaplan-Meier product limit estimate curves. Statistical significance was determined using Cox-Tarone binary regression-based tests and Gehan-Breslow nonparametric score-based tests. Palpable tumors were analyzed by life table technique (log rank test). Occult or internal tumors were classified as fatal or incidental. Exact permutation test was then used to test tumor incidence rates. When the number of tumor occurrences was more than eight, Peto's mortality-prevalence test was used to analyze the tumor data. Rare tumors were tested at the 5%, one-tailed probability level, and common tumors at the 1%, one-tailed probability level.

Observations and times:

Clinical signs: The animals were observed twice daily for mortality and moribundity. In addition, detailed observations were performed once prior to initiation of treatment and once a week during the study.

Body weights: The body weights were recorded prior to treatment, weekly for Weeks 1-26, once every other week from week 26-52, and once every 4 weeks thereafter.

Food consumption: Individual food consumption was recorded weekly for Weeks 1 through 26, once every other week during weeks 26-52, and once every 4 weeks thereafter.

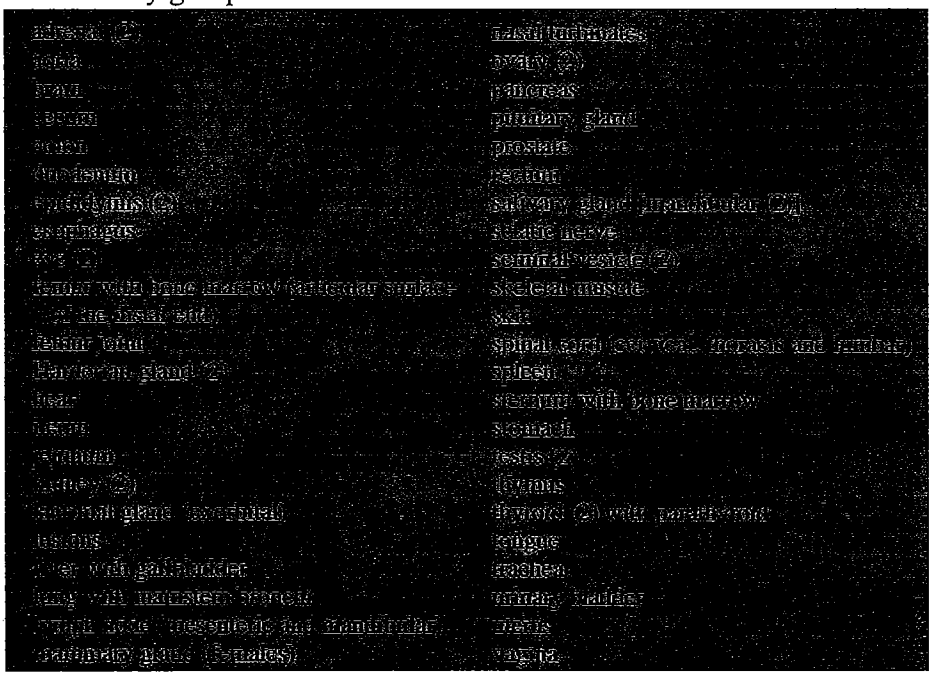
Hematology: Blood samples for hematological analyses were collected prior to sacrifice during Weeks 105 and 106.

Clinical chemistry: Blood samples for clinical chemistry analyses were collected prior to sacrifice during Weeks 105 and 106.

Organ weights: The weights of the following organs were recorded: adrenal, brain, epididymis, heart, intestine (cecum, colon), kidney, liver with gall bladder, lungs, ovary, pituitary, prostate, spleen, stomach, testes, thyroid and uterus.

Gross pathology: At the end of the dosing period, the surviving animals from the main study groups were euthanized and complete necropsies performed. Necropsy was also conducted on all animals that died or sacrificed unscheduled during the dosing period.

Histopathology: Histopathological examinations of the following organs from all main study group animals were conducted.



Toxicokinetics: Blood samples for toxicokinetic analysis were collected from the satellite animals (3 animals/sex/group) on Day 1 and Day 26 at 0.5, 1, 2, 6 and 24 hours after dosing.

Results:

Mortality: Survivals of animals in the treatment groups were similar to that of controls. A total of 38, 42, 35, 32 and 42 male mice, and 40, 36, 43, 32 and 33 female mice survived to terminal sacrifice in the 0, 25, 75, 200 and 500 µg/kg/day groups, respectively. Mortality and survival rate data are summarized in the Tables and Figures below.

Table: Cumulative mortalities of **male** mice receiving different doses of RU-0211 for 104 weeks.

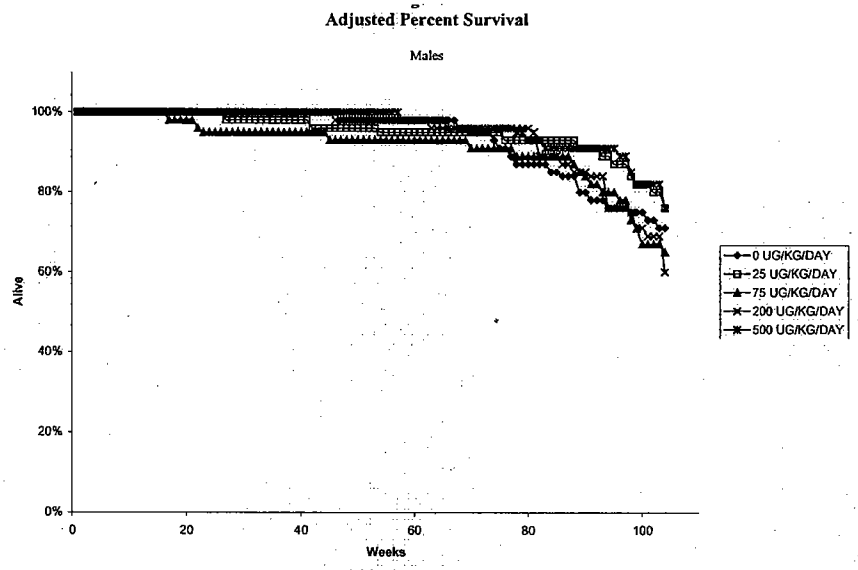
Weeks	Control		25 µg/kg/day		75 µg/kg/day		200 µg/kg/day		500 µg/kg/day	
	X/N	%	X/N	%	X/N	%	X/N	%	X/N	%
13	0/55	0	0/55	0	0/55	0	0/55	0	0/55	0
26	0/55	0	0/55	0	3/55	5	0/55	0	0/55	0
52	1/55	2	2/55	4	4/55	7	1/55	2	0/55	0
78	6/55	11	4/55	7	6/55	11	2/55	4	3/55	5
104	15/55	27	13/55	24	19/55	35	22/55	40	13/55	24

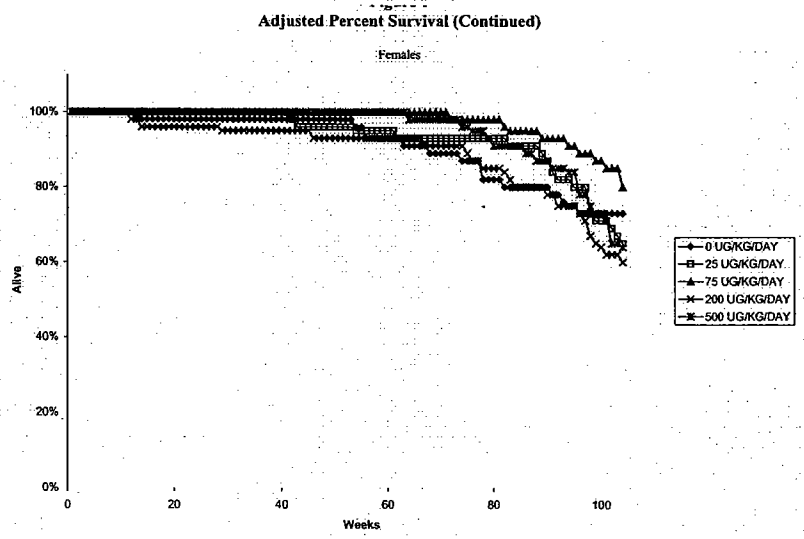
X, number of animals found dead or sacrificed at stated time; N, total number of animals.

Table: Cumulative mortalities in **female** mice receiving different doses of RU-0211 for 104 weeks.

Weeks	Control		25 µg/kg/day		75 µg/kg/day		200 µg/kg/day		500 µg/kg/day	
	X/N	%	X/N	%	X/N	%	X/N	%	X/N	%
13	1/55	2	0/55	0	0/55	0	1/55	2	0/55	0
26	1/55	2	0/55	0	0/55	0	2/55	4	0/55	0
52	1/55	2	2/55	4	0/55	0	4/55	7	0/55	0
78	10/55	18	4/55	7	1/55	2	8/55	15	3/55	6
104	15/55	27	19/55	35	11/55	20	21/55	38	20/55	36

X, number of animals found dead or sacrificed at stated time; N, total number of animals.



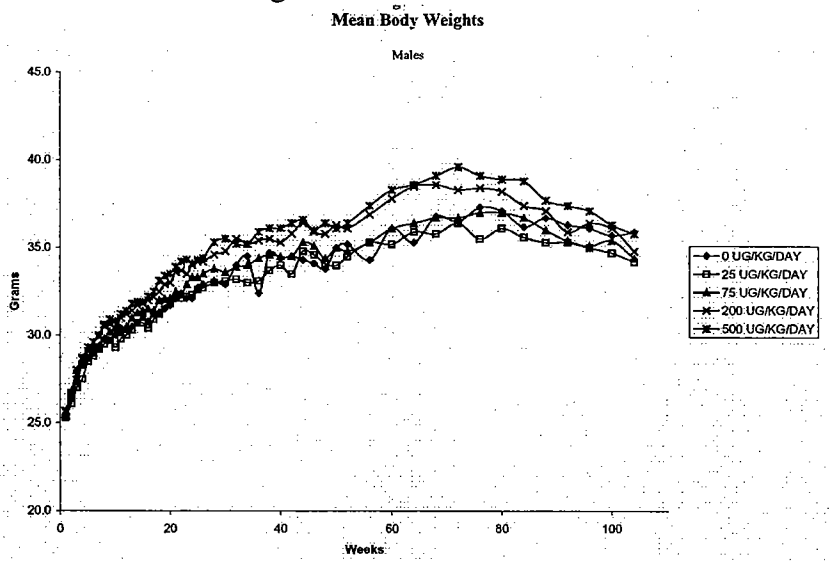


Clinical signs: No treatment-related clinical signs were observed in any group.
Body weights: The mean body weights of the control male and female mice were 25.3±2.02 g and 19.2±1.08 g, at the beginning of the dosing period (Week 1) and

35.9±2.74 g and 32.9±3.91 g in Week 104, respectively. The mean body weights of 200 and 500 mg/kg group males were slightly higher (4.5% to 8.5%) than that of controls from week 13 to week 72. The mean body weights of the high dose females were slightly higher (2.4% to 4.6%) than that of controls from week 9 to week 56. However, at the end of the dosing period, no differences in the body weights were observed between the control and treatment group male and female mice. The body weights of the male and female animals at pre-dose and Week 105 are shown in the Table below.

Weeks	Males					Females				
	Control	25	75	200	500	Control	25	75	200	500
Week 1										
Body Weight (g)	25.3	25.3	25.4	25.6	25.7	19.2	18.7	19.2	19.5	19.4
% of control (C1)		100	100.4	101.2	101.6		97.4	100	101.6	101.0
Week-105										
Body Weight (g)	35.9	34.2	34.4	34.8	35.8	32.9	32.2	32.6	31.9	33.9
% of control (C1)		95.3	95.8	96.9	99.7		97.9	99.1	97.0	103.0

The body weights (g) of control and treatment group male and female mice during the dosing period are shown in the Figures below.



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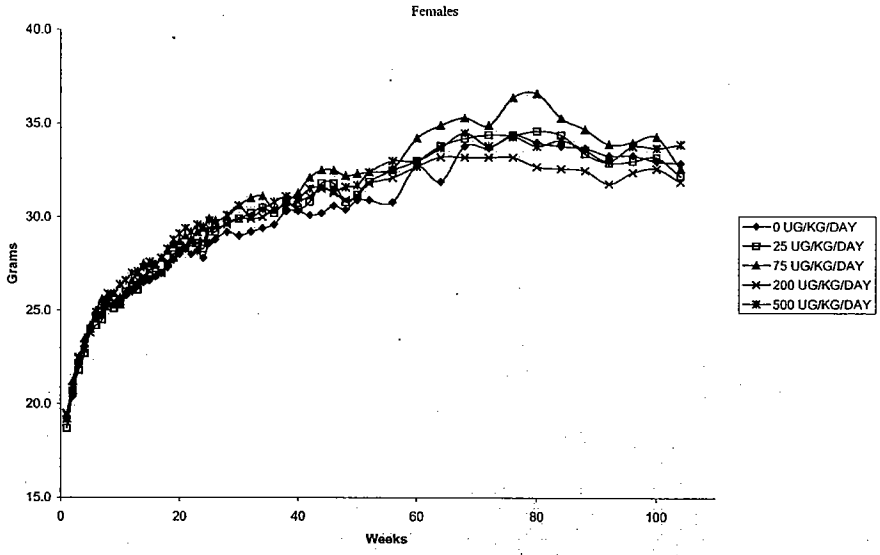
ON ORIGINAL

Mean Body Weights (Continued)



Food consumption: The mean food consumptions of the control male and female animals in Week 1 were 6.5 and 5.7 g/animal/day, respectively. Males receiving 200 and 500 µg/kg/day doses of RU-0211 had slightly higher food consumptions during most part of the dosing period. Females receiving 75, 200 and 500 mg/kg doses had intermittent slight increases in food consumptions during parts of the dosing period.

Hematology: No treatment-related changes in hematological parameters were observed in any group.

Gross pathology: Males and females receiving the high dose had thickened mucosa of the glandular stomach (0/55, 0/55, 0/55, 1/55 and 3/55 in control, 25, 75, 200 and 500 µg/kg/day doses, respectively, in both males and females).

Organ Weights: The weights of the stomach of treatment group male and female mice were higher than that of controls. The mean relative stomach weights of the male animals were 9.1, 21.8, 26.1 and 37.3% higher than that of control, respectively. The mean relative stomach weights of the female animals were 13.4, 22.5, 30.0 and 35.5% higher than that of controls at 25, 75, 200 and 500 µg/kg/day doses, respectively.

Histopathology:

Nonneoplastic: Males receiving the high dose had higher incidences of inflammation of the lung. Treatment group males had significantly higher incidences of hyperplasia of the epithelium of the nonglandular and glandular stomach. Males treated with 200 and 500 µg/kg doses had higher incidences of hyperplasia of the marginal plate of the nonglandular stomach, and the 500 µg/kg males had higher incidences of adenomatous focal hyperplasia of the glandular stomach. Treatment group females had higher incidences of epithelial hyperplasia and hyperplasia of the marginal plate of the nonglandular and glandular stomachs. High dose females had bronchioloalveolar hyperplasia and focal osseous metaplasia of the kidney. The nonneoplastic changes in male and female mice are shown in the Table below.

Histopathological Lesion	Control	25 µg/kg	75 µg/kg	200 µg/kg	500 µg/kg
MALES					
Lung					
-Inflammation, focal	0/55	1/55	1/55	1/55	3/55
Kidney					
-Cyst	1/55	2/55	2/55	8/55	7/55
-Cortical scar	0/55	1/55	1/55	4/55	3/55
Stomach, nonglandular					
-Hyperplasia, marginal plate	9/55	4/55	8/55	14/55	16/55
-Hyperplasia, epithelial	1/55	2/55	8/55	5/55	12/55
Stomach, glandular					
-Hyperplasia, mucosal epithelium	2/55	2/55	13/55	9/55	19/55
-adenomatous hyperplasia, focal	0/55	0/55	0/55	1/55	4/55
-dilated gland	0/55	2/55	5/55	4/55	4/55
Eye					
-Harderian gland, focal hyperplasia	0/55	2/55	0/55	0/55	2/55
FEMALES					
Lung					
-bronchioloalveolar hyperplasia	1/55	0/55	2/55	2/55	3/55
Kidney					
-Osseous metaplasia, focal	0/55	1/55	2/55	1/55	3/55
Eye					
-Harderian gland, focal hyperplasia	0/55	1/55	2/55	0/55	1/55
Stomach, nonglandular					
-Hyperplasia, marginal plate	3/55	8/55	14/55	17/55	12/55
-Hyperplasia, epithelial	1/55	4/55	18/55	12/55	26/55
-Keratin cyst	0/55	0/55	2/55	0/55	4/55
Stomach, glandular					
-Hyperplasia, mucosal epithelium	3/55	15/55	20/55	17/55	13/55
-adenomatous hyperplasia, focal	0/55	0/55	0/55	0/55	1/55
-hyperplasia, marginal plate	0/55	2/55	5/55	2/55	0/55

Neoplastic: Following oral administration of RU-0211 to female mice for 104 weeks, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%); p=0.0493, trend test]. A positive trend was also observed for the combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122, sponsor; p=0.0162, CDER Statistician] in female mice. There was no positive trend for Harderian gland tumors in male mice. The tumor incidences in male and female mice are shown in the Table below.

Organ/tumor	Control	25 µg/kg	75 µg/kg	200 µg/kg	500 µg/kg	P value (Trend test)
MALES						
Eye						
Harderian gland						
-adenoma	1/55 (1.8%)	3/55 (5.5%)	3/55 (5.5%)	4/55 (7.3%)	4/55 (7.3%)	0.1745
-Carcinoma	1/55 (1.8%)	2/55 (3.6%)	0/55 (0%)	1/55 (1.8%)	1/55 (1.8%)	--
-Adenoma+ carcinoma	2/55 (3.6%)	5/55 (9.1%)	3/55 (5.5%)	5/55 (9.1%)	6/55 (10.9%)	0.2363
FEMALES						
Eye						
Harderian gland						
-adenoma	2/55 (3.6%)	2/55 (3.6%)	2/55 (3.6%)	1/55 (1.8%)	5/55 (9.1%)	0.0586
-Carcinoma	0/55 (0%)	0/55 (0%)	1/55 (1.8%)	2/55 (3.6%)	2/55 (3.6%)	0.0493
-Adenoma+ carcinoma	2/55 (3.6%)	2/55 (3.6%)	3/55 (5.5%)	3/55 (5.5%)	7/55 (12.7%)	0.0122

Toxicokinetics: Toxicokinetic analysis on Day 1 and during Week 26 showed that plasma concentrations of RU-0211 in the 25 µg/kg/day group males and females were below the lower limit of detection. The mean maximum plasma concentrations of RU-0211 was reached in 0.5 to 1 hr, and reached below the lower limit of detection in 24 hours after dosing. The mean C_{max} and $AUC_{0\text{-}24hr}$ values increased with increasing doses from 75 µg/kg to 500 µg/kg. No differences in the exposure levels were observed between male and female mice, and there was no accumulation of the drug following repeated oral administration. Toxicokinetic parameters for RU-0211 in male and female mice are summarized in the Table below.

Summary of Toxicokinetic Data

Sex/Group µg/kg/day	Male 6 25	Male 7 75	Male 8 200	Male 9 500	Female 6 25	Female 7 75	Female 8 200	Female 9 500
C_{max} (pg/mL)								
Day 1	NC	144	235	625	NC	161	500	765
Week 26	NC	NC	280	328	NC	179	216	645
T_{max} (hr)								
Day 1	NC	0.500	0.500	0.500	NC	0.500	0.500	0.500
Week 26	NC	NC	1.00	0.500	NC	0.500	1.00	0.500
$AUC_{0\text{-}24hr}$ (pg·hr/mL)								
Day 1	NC	837	387	1850	NC	80.5	305	2040
Week 26	NC	NC	279	1620	NC	89.5	485	3260

NC not calculated
N 3

Summary of individual study findings:

Male mice, treated with RU-0211 had significantly higher incidences of hyperplasia of the epithelium of the nonglandular and glandular stomach. In addition, male animals treated with 200 and 500 µg/kg doses had higher incidences of hyperplasia of the marginal plate of the nonglandular stomach, and the high dose males had higher incidences of adenomatous focal hyperplasia of the glandular stomach. Treatment group females had higher incidences of epithelial hyperplasia and hyperplasia of the marginal plate of the nonglandular and glandular stomachs. However, no significant increase in tumor incidences in the stomach was observed in male and female mice.

In female mice, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%);

p=0.0493, trend test]. A positive trend was also observed for combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122, sponsor, p=0.0162, CDER statistician; trend test] in female mice.

Adequacy of the carcinogenicity study and appropriateness of the test model: The dose selection for the 2-year mouse carcinogenicity study was appropriate, and concurred by the CDER Executive CAC Committee. The conduct of the study was appropriate and acceptable.

Evaluation of tumor findings: In female mice, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%); p=0.0493, trend test]. A positive trend was also observed for combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122, sponsor; p=0.0162, CDER statistician, trend test] in female mice. However, according to the CDER standard, to be statistically significant, the p values for the common tumors should be less than 0.005. Thus, according to the CDER criteria, the incidences of Harderian gland adenoma + carcinoma in female mice were not significant.
The incidences of Harderian gland carcinoma at 200 and 400 µg/kg doses in female mice were higher than the historical control incidences from the conducting laboratory in this strain of mice (0% to 2%; mean, 0.67%). The incidences of Harderian gland carcinoma in female mice were also higher than the background incidences in this strain of mice (0% to 2%, mean 1.3%; NTP database).
The plasma exposure levels of RU-0211 in mice could not be compared with the exposure levels in humans, as the plasma level of the parent compound in humans was below the limit of detection following administration of the clinical dose of 48 µg/day (24 µg b.i.d). The doses of 25, 75, 200 and 500 µg/kg in mice are approximately 2, 6, 17 and 42 times the human dose of 48 µg/day (on the basis of body surface area).

Carcinogenicity conclusions:

In the 2-year oral carcinogenicity study with RU-0211 in mice, groups of animals received 0, 25, 75, 200 and 500 µg/kg/day doses of the drug. The doses for the mouse carcinogenicity study were concurred by the CDER Executive CAC Committee. Treatment with RU-0211 had no significant effect on the survival of male and female mice. At the end of the treatment period, no treatment-related changes in the body weights were observed in any group.
In female mice, there was a positive trend for Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%); p=0.0493, trend test]. A positive trend was also observed for combined incidences of Harderian gland adenoma and carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%); p=0.0122, sponsor; p=0.0162, CDER statistician, trend test] in female mice. However, according to the CDER standard, to be statistically significant, the p values for the common tumors should be less than 0.005. Thus, according to the CDER criteria, the incidences of Harderian gland adenoma + carcinoma in female mice were not significant.

The incidences of Harderian gland carcinoma at 200 and 400 µg/kg doses in female mice were higher than the historical control incidences from the conducting laboratory for this tumor in this strain of mice (0% to 2%; mean, 0.67%) as well as the background incidences in this strain of mice (0-2%; mean, 1.3%; NTP database).

Recommendations for further analysis: None.

Labeling recommendations: None.

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

P/T REVIEWER(s): Sushanta Chakder, Ph.D.
DATE: 12/12/05
IND/NDA: NDA 21-908
DRUG CODE#: RU-0211/SPI-0211
CAS#: 136790-76-6, 333963-40-9
DIVISION(s): Division of Gastroenterology Products, HFD-180
DRUG NAME(s): Lubiprostone
SPONSOR: Sucampo Pharmaceuticals, Inc., Bethesda, MD.
LABORATORY: —
CARCINOGENICITY STUDY COMPLETION DATE: February 14, 2005
THERAPEUTIC CATEGORY: Laxative.
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Prostaglandin Analog.
MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): RU-0211 was not genotoxic in a battery of genotoxicity assays, including the Ames test, the *in vitro* chromosome aberration assay in Chinese hamster lung (CHL) cells, the *in vitro* mouse lymphoma cell forward gene mutation assay and the *in vivo* mouse bone marrow micronucleus assay.

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.):

RAT STUDY DURATION (weeks): 104 Weeks
STUDY STARTING DATE: January 17, 2002
STUDY ENDING DATE: February 23, 2005
RAT STRAIN: CD® (SD)IGS BR VAF/Plus rats
ROUTE: Oral gavage
DOSING COMMENTS: None
NUMBER OF RATS:

- Control-1 (C1): 65 animals/sex
- Low Dose (LD): 65 animals/sex
- Middle Dose (MD): 65 animals/sex
- High Dose (HD): 65 animals/sex

RAT DOSE LEVELS* (µg/kg/day):

- Low Dose: 20 µg/kg/day
- Middle Dose: 100 µg/kg/day
- High Dose-1: 400 µg/kg/day

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): The doses for the 2-year rat carcinogenicity study were selected on the basis of toxicity endpoints and the MTD from a 26-week oral toxicity with RU-0211 in Sprague-Dawley rats. In the 26 toxicity study in rats, The MTD was 400 µg/kg/day in males, and >400 µg/kg/day in females. This was based on decreased body weights in male (8.1%) and female (3.3%) animals. In addition, in a 4-week oral toxicity study in rats, diarrhea was observed at 1000 mg/kg/day in both males and females. Based on these findings, the 400 µg/kg dose was selected as the high dose for the carcinogenicity study in rats. The doses of 20, 100 and 400 µg/kg/day were concurred by the CDER Executive CAC Committee (November 27, 2001; see Appendix 3).

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): The doses for the 2-year carcinogenicity study in rats were concurred by the CDER Executive CAC Committee (November 27, 2001; see Appendix 3).

RAT CARCINOGENICITY (conclusion: negative; positive; MF; M; F): RU-0211 was carcinogenic in male and female rats.

RAT TUMOR FINDINGS: In male rats, a positive trend for the incidences of squamous cell papilloma in the nonglandular stomach [control, 1/65 (1.5%); low dose, 1/65 (1.5%), mid dose, 5/65 (7.7%), high dose, 6/64 (9.4%); p=0.0135, trend test] was observed. A positive trend was also observed for the combined incidences of squamous cell papilloma and carcinoma in male rats [control, 2/65 (3.1%); low dose, 1/65 (1.5%), mid dose, 6/65 (9.2%), high dose, 7/64 (10.9%); p=0.0156, trend test]. However, according to the CDER statistical standard, to be significant, the p values for the common tumors should be <0.005. Thus, according to the CDER criteria, the incidences of squamous cell papilloma + carcinoma in the non-glandular stomach of male rats were not significant. The incidences of histiocytic sarcoma [control, 6/65 (9.2%); low dose, 1/65

(1.5%), mid dose, 4/65 (6.2%), high dose, 10/65 (15.4%); p=0.0015, sponsor; p=0.0091, CDER statistician; trend test] and benign interstitial cell tumor of the testes [control, 2/65 (3.1%); low dose, 4/65 (6.2%), mid dose, 1/65 (1.5%), high dose, 10/65 (15.4%); p=0.0002, sponsor; p=0.0006, CDER statistician; trend test] were significantly higher in high dose male rats. Although, there was a positive trend for the incidences of histiocytic sarcoma in male rats, the incidences of this tumor at any dose were not significantly different from the control incidences.
The incidences of squamous cell papilloma and combined incidences of papillloma and carcinoma in the nonglandular stomach of male rats were higher than historical control incidences (squamous cell papilloma- 0% – 1.5%, mean 0.66%; papilloma+carcinoma – 0% to 3%, mean 1.42%) in this strain of rat from the conducting laboratory. The incidences of histiocytic sarcoma and interstitial cell tumor of the testes in male rats were also higher than the conducting laboratory's historical control incidences for these tumors in this strain of rat (histiocytic sarcoma – 0% to 4.3%, mean 0.87%; interstitial cell tumor of testes – 0% to 8.3%, mean 3.2%). The incidences of testicular interstitial cell tumors in male rats at the high dose were higher than the background incidences in this strain of rat (1.43% to 7.14%; mean, 2.35%; Charles River Laboratories).
In female rats, a positive trend for the incidences of hepatocellular adenoma [control, 0/65 (0%); low dose, 0/65 (0%), mid dose, 1/65 (1.5%), high dose, 5/65 (7.7%); p=0.0012, sponsor; p=0.0031, CDER statistician; trend test] was observed. The incidences of hepatocellular adenoma in female rats were higher than the historical control incidences for this tumor from the conducting laboratory (0% to 1.5%, mean 0.8%). The incidences of hepatocellular adenoma in female rats were higher than the mean background incidences in this strain (2.02%; range, 0.77% to 13.3%; Charles River Laboratories).

RAT STUDY COMMENTS: None

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. **Study No.:** 7142-100
2. **Name of Laboratory:** —
3. **Strain:** .CD®(SD) IGS BR VAF/Plus rats
4. **No./sex/group:** 65/sex/group
5. **Doses (0, L, M, and H):** 0, 20, 100 and 400 µg/kg/day

Basis for dose selection stated. Yes. The dose selection was based on the MTD from a 26-week oral toxicity study with RU-0211 in Sprague-Dawley rats. In the 26-week oral toxicity study in rats, the MTD was 400 µg/kg/day in males and >400 µg/kg/day in females. In a 4-week oral toxicity study in rats, diarrhea was observed in both males and females at 1000 µg/kg/day. The 20, 100 and 400 µg/kg/day doses of RU-0211 for the 2-year rat carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001).

6. **Interim sacrifice:** No
7. **Total duration (weeks):** 104 Weeks
8. **Week/site for first tumor:**

	Male	**Female**
Control	47/Hemapoietic neoplasia (lung)	50/Mammary carcinoma
Low dose	31/Oligodandroglioma (brain)	47/Mammary carcinoma
Mid dose	32/Pituitary adenoma	21/Nephroblastoma
High dose	59/Osteosarcoma (head)	59/Pancreatic acinar cell carcinoma

9. **Number alive at termination:**

	Male	**% survival**	**Female**	**% survival**
Control 1	36/65	55%	21/65	32%
Low dose	44/65	68%	24/65	37%
Mid dose	35/65	54%	26/65	40%
High dose	33/65	51%	35/65	54%

Statistical methods used: One-way analysis of variance (ANOVA) was used to analyze the effects of the drug on body weights, food consumption, and hematology parameters. If ANOVA on homogenous or transformed data was significant, Dunnett's multiple comparison t-test was used for pair wise comparisons between treated and control groups. Graphical evaluation of survival was performed using Kaplan-Meier product limit estimate curves. Statistical significance was determined using Cox-Tarone binary regression-based tests and Gehan-Breslow nonparametric score-based tests. Palpable tumors were analyzed by life table technique (log rank test). Occult or internal tumors were classified as fatal or incidental. Exact permutation test was then used to test tumor incidence rates. When the number of tumor occurrences was more than eight, Peto's mortality-prevalence test was used to analyze the tumor data. Rare tumors were tested at the 5%, one-tailed probability level, and common tumors at the 1%, one-tailed probability level.

10. **Attach tumor and non-tumor data for each tissue:** See Appendix 2.

Study title: **104-Week oral gavage carcinogenicity study with RU-0211 in rats**

Key study findings: Male rats receiving RU-0211 had significantly higher incidences of squamous cell papilloma in nonglandular stomach. The combined incidences of squamous cell papilloma and carcinoma in male rats were also higher than control. There was a positive trend for the incidences of histiocytic sarcoma and benign interstitial cell tumor of the testes in male rats. Treatment of the female rats with RU-0211 produced hepatocellular adenoma at 100 and 400 µg/kg doses.

Study number: 7142-100
Volume #, and page #: The study report was submitted electronically.
Conducting laboratory and location:
Date of study initiation: January 17, 2002.
Date of study report: February 23, 2005.
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: RU-0211, Lot # 020108, #020109, #020702, #020924, purity,

CAC concurrence: Yes.

Study Type: 2-year rodent carcinogenicity study
Species/strain: CD® (SD)IGS BR VAF/Plus rats
Number/sex/group; age at start of study: 65/sex/dose group; at initiation of treatment, the animals were approximately 49 days old; body weights- Males 199 to 263 g and Females 147 to 209 g.
Animal housing: Animals were housed individually in suspended, wire mesh stainless-steel cases.
Formulation/vehicle: RU-0211, dissolved in 1% Polysorbate 80 solution (in water), was used in the study. Control animals received the vehicle (1% Polysorbate 80 solution).
Drug stability/homogeneity: Duplicate samples of the dosing solutions were analyzed by the sponsor, and were found to be stable.

Methods:

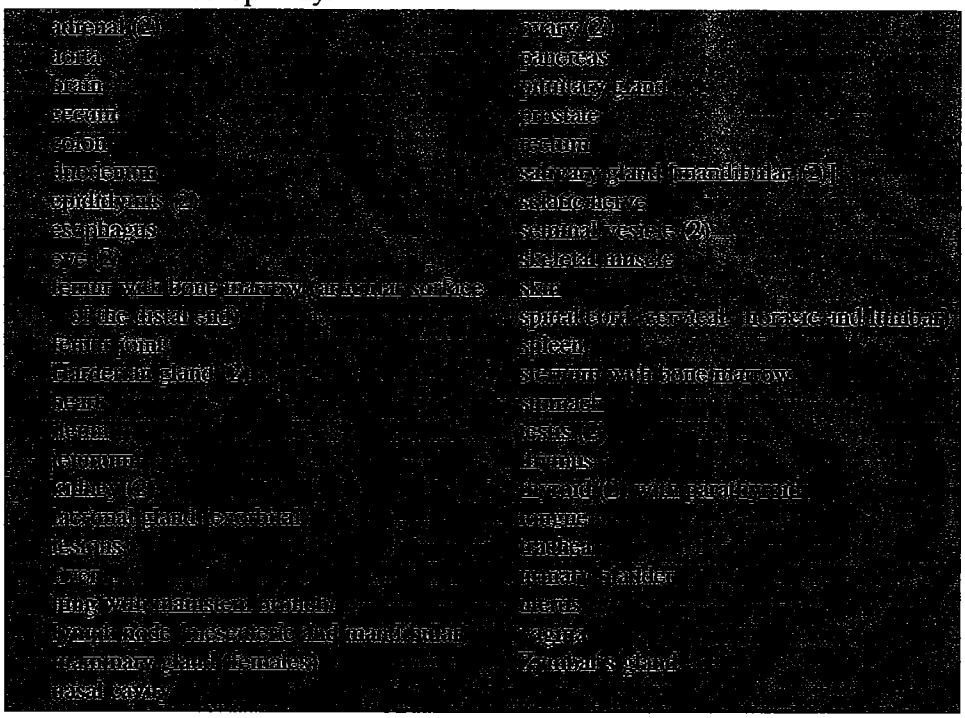
Doses: The doses of RU-0211 used in the rat carcinogenicity study were 20, 100 and 400 µg/kg/day (5 ml/kg).
Basis of dose selection: The dose selection for the 2-year rat carcinogenicity study was based on the MTD from a 26-week oral toxicity study with RU-0211 in Sprague-Dawley rats. In the 26-week oral toxicity study in rats, the MTD was 400 µg/kg/day for males and >400 µg/kg/day for females. However, as diarrhea was observed at the 1000 µg/kg/day in a 4-week oral toxicity study in rats, 400 µg/kg/day was selected as the high dose for both male and female rats. The low and mid doses were selected as 20 and 100 µg/kg/day. The doses for the rat carcinogenicity study were concurred by the CDER Executive CAC Committee (November 27, 2001; see Appendix 3).
Restriction paradigm for dietary restriction studies: None

Route of administration: The drug was administered by oral gavage.
Frequency of drug administration: The doses were administered once a day at a dosing volume of 5 ml/kg.
Dual controls employed: No
Interim sacrifices: No
Satellite PK or special study group(s): For toxicokinetic analysis, satellite groups consisting of 9/rats/sex/group were administered 10, 100 and 400 µg/kg/day doses of the drug. Blood samples were collected from 3 rats/sex/group on Day 1 and Week 26 at 0.5, 1, 2, 6 and 24 hours after dosing.
Deviations from original study protocol: None.
Statistical methods: One-way analysis of variance (ANOVA) was used to analyze the effects of the drug on body weights, food consumption, and clinical pathology parameters. If ANOVA on homogenous or transformed data was significant, Dunnett's multiple comparison t-test was used for pairwise comparisons between treated and control groups. Adjusted survival data for carcinogenicity animals were analyzed by the National Cancer Institute (NCI) life table package consisting of Kaplan-Meier product limit estimation curves. Non-neoplastic lesions were analyzed by the Cochran-Armitage test for trend and Fischer-Irwin exact test. Incidental tumors were analyzed by logistic regression of tumor tests, and the palpable tumors were analyzed in the same manner as survival, using the first palpation tome as the tumor onset time. Occult or internal tumors were classified as fatal or incidental. When the number of tumor occurrences was more than eight, Peto's mortality-prevalence test was used to analyze the tumor data. Rare tumors were tested at the 5%, one-tailed probability level, and common tumors at the 1%, one-tailed probability level.

Observations and times:

Clinical signs: The animals were observed twice daily for mortality and moribundity. In addition, detailed observations for abnormality and signs of toxicity were performed once a week.
Body weights: The body weights were recorded prior to treatment, weekly for Weeks 1-26, once every other week during Weeks 26-51, and once every 4 weeks thereafter.
Food consumption: Individual food consumption was measured prior to initiation of treatment, weekly for Weeks 1-26, once every other week during Weeks 26-51, and once every 4 weeks thereafter.
Hematology: Blood samples for hematological analyses were collected at termination.
Clinical chemistry: Blood samples for clinical chemistry analyses were collected at termination.
Organ weights: The weights of the following organs were measured at scheduled sacrifice: adrenal, brain, epididymis, heart, cecum and colon, kidney, liver, lung, prostate, spleen, stomach, testes, thymus, thyroid, uterus.
Gross pathology: At the end of the 104-week treatment period, all surviving animals were euthanized and complete necropsies performed. Necropsy was also performed on all animals that died or sacrificed unscheduled during the dosing period.

Histopathology: The following tissues from all animals were preserved in 10% neutral-buffered formalin for histopathological examinations. Tissues from all groups of rats were examined microscopically.



Toxicokinetics: Blood samples for toxicokinetic analysis were collected from the satellite group animals (3 animals/sex/group) on Day 1 and Week 26 at 0.5, 1, 2, 6 and 24 hours after dosing.

Results:

Mortality: Statistical analysis of the adjusted mortality showed that there were significantly lower mortalities in 20 µg/kg/day males and 400 µg/kg/day females. At the end of the dosing period, there were 36, 44, 35 and 33 surviving males and 21, 24, 26 and 35 surviving females in the control, 20, 100 and 400 µg/kg/day doses, respectively. Cumulative mortalities among male and female rats at different times after dosing are shown in the Tables and Figures below.

Table: Cumulative mortalities in **male** rats receiving different doses of RU-0211 for 104 weeks.

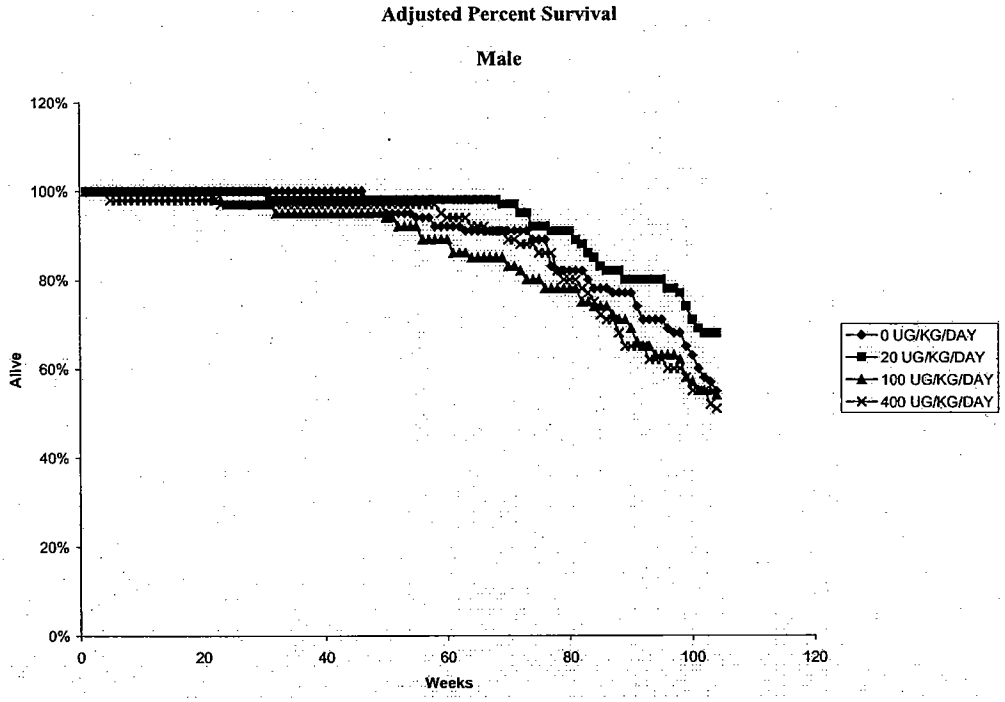
Weeks	Control		20 µg/kg/day		100 µg/kg/day		400 µg/kg/day	
	X/N	%	X/N	%	X/N	%	X/N	%
13	0/65	0	0/65	0	0/65	0	1/65	1.5
26	0/65	0	0/65	0	2/65	3.1	2/65	3.1
52	3/65	4.6	1/65	1.5	5/65	7.7	2/65	3.1
78	12/65	18.5	6/65	9.2	14/65	21.5	11/65	17.0
104	29/65	44.4	21/65	32.3	30/65	46.2	32/65	49.2

X, number of animals found dead or sacrificed at stated time; N, total number of animals.

Table: Cumulative mortalities in **female** rats receiving different doses of RU-0211 for 104 weeks.

Weeks	Control		20 µg/kg/day		100 µg/kg/day		400 µg/kg/day	
	X/N	%	X/N	%	X/N	%	X/N	%
13	0/65	0	0/65	0	1/65	1.5	0/65	0
26	0/65	0	0/65	0	2/65	3.1	0/65	0
52	1/65	1.5	2/65	3.1	3/65	4.6	0/65	0
78	6/65	9.2	12/65	18.5	10/65	15.4	6/65	9.2
104	44/65	67.7	41/65	63.1	39/65	60	30/65	46.2

X, number of animals found dead or sacrificed at stated time; N, total number of animals.



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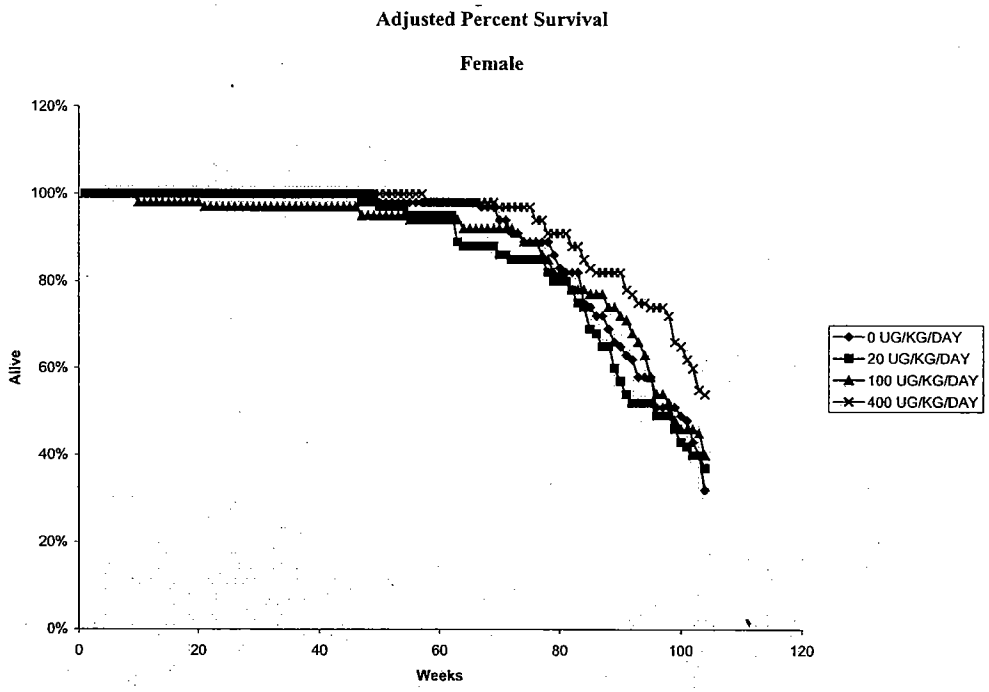

Clinical signs: Treatment group males and females had higher incidences of clear discharge from the mouth and slightly higher incidences of alopecia. The incidences of oral discharge and alopecia in male and female animals are shown in the Table below.

Clinical Sign	Control		Low dose		Mid dose		High dose	
	Male	Female	Male	Female	Male	Female	Male	Female
Oral discharge (clear)	4/65	2/65	16/65	7/65	24/65	22/65	43/65	28/65
Alopecia	4/65	9/65	7/65	12/65	5/65	15/65	10/65	14/65

Body weights: The mean body weights of the control male and female animals at the beginning of the dosing period (Week 1) were 232±12.5 g and 177±11.4 g, and in Week 104 were 591±95.3 g and 409±76.9 g, respectively. The mean body weights of the mid and high dose males were slightly lower than that of controls during most part of the treatment period. The mean body weights of the females were similar in all groups. The body weights and changes of body weights from controls for the male and female animals during different times of the dosing period are shown in the Table and Figures below.

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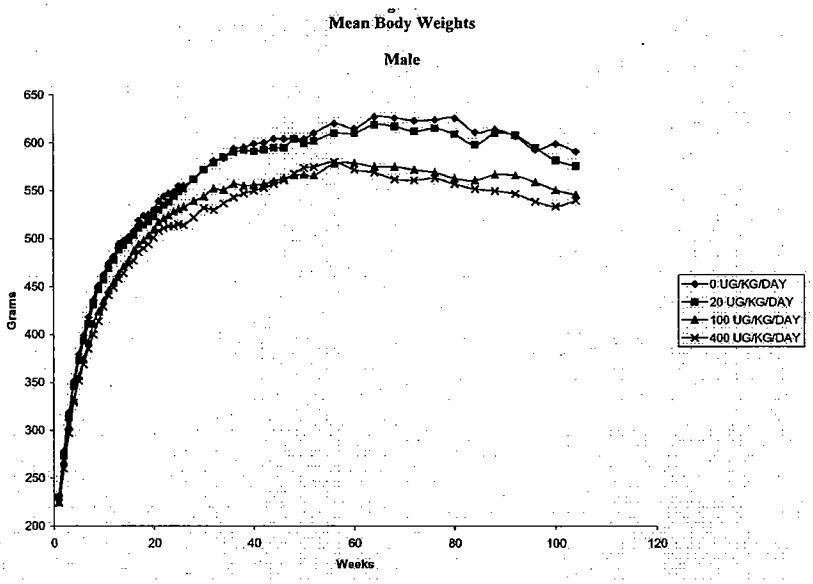
Weeks	Males				Females			
	Control	Low	Mid	High	Control	Low	Mid	High
Week-13								
Body Weight (g)	494	499	463*	459*	274	279	279	296
% of Control	**100%**	**101%**	**94%**	**93%**	**100%**	**102%**	**102%**	**108%**
Week-26								
Body Weight (g)	555	552	533	514*	299	309	306	305
% of Control	**100%**	**99%**	**96%**	**93%**	**100%**	**103%**	**102%**	**102%**
Week-52								
Body Weight (g)	610	602	566*	575	349	358	359	356
% of Control	**100%**	**99%**	**93%**	**94%**	**100%**	**103%**	**103%**	**102%**
Week-90								
Body Weight (g)	626	609	563*	557*	399	398	407	393
% of Control	**100%**	**97%**	**90%**	**89%**	**100%**	**100%**	**102%**	**99%**
Week-104								
Body Weight (g)	591	576	546	540	409	396	399	404
% of Control	**100%**	**97%**	**92%**	**91%**	**100%**	**97%**	**98%**	**99%**

*Significantly ($p<0.05$) decreased compared to control.



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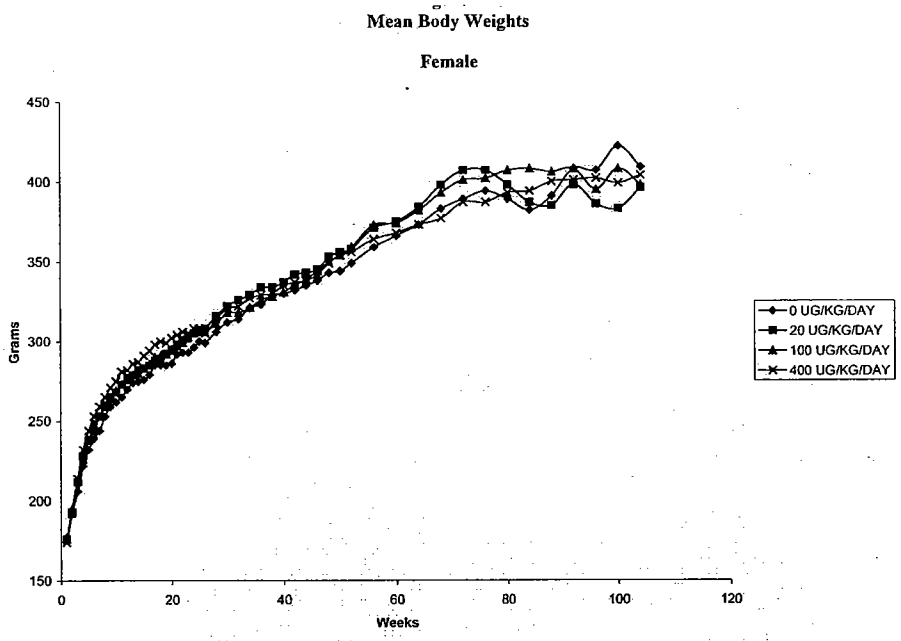

Food consumption: The mean food consumptions of the control male and female animals in Week 1 were 25.7 and 16.5 g/animal/day, respectively. Males and females receiving RU-0211 had higher food intakes in most part of the dosing period. The food consumption in males increased (4.2% to 17.4%) consistently only at the high dose, and in females, significant increases were observed at the mid (4% to 15%) and the high (9% to 21%) dose.

Hematology: No treatment-related effects on hematological parameters were observed.

Gross pathology: Males and females treated with RU-0211 had higher incidences of enlarged adrenal glands, distended nonglandular stomach, and mucosal thickening of the glandular stomach. There were higher incidences of enlarged spleen, thickening of the wall of the cecum and enlarged mesenteric lymph nodes in high dose males and females. High dose females had higher incidences of uterine cyst, and treatment group females had thickened wall of the uterus. Gross pathological changes observed in male and female rats are shown in the Table below.

Organ/finding	MALES				FEMALES			
	Control	Low	Mid	High	Control	Low	Mid	High
Adrenal, cortex -enlarged	1/65	1/65	2/65	2/65	1/65	6.65	8/65	11/65
Lung -pale area	5/65	3/65	6/65	5/65	1/65	2/65	4/65	6/65
Spleen -enlarged	3/65	0/65	0/65	8/65	2/65	0/65	0/65	4/65
Kidney -pale area	1/65	0/65	1/65	4/65	0/65	0/65	0/65	0/65
Stomach, nonglandular -Distended	1/65	2/65	5/65	2/65	0/65	1/65	3/65	2/65
-wall thickened	1/65	2/65	1/65	2/65	0/65	0/65	0/65	2/65
Stomach, glandular -mucosa thickened	2/65	28/65	34/65	39/65	0/65	8/65	24/65	38/65
Cecum -wall thickened	0/65	0/65	0/65	2/65	0/65	0/65	0/65	2/65
Mesenteric lymph nodes -enlarged	0/65	2/65	1/65	6/65	0/65	0/65	0/65	2/65
Utereus - Cyst	--	--	--	--	3/65	2/65	2/65	8/65
-wall thickened					0/65	1/65	3/65	3/65

Organ Weights: The relative (to body weight) weights of the brain (5.3% and 13.6% at mid and high doses, respectively), liver (20.7%, 18.8% and 34.6% at low, mid and high doses, respectively), kidneys (11.5%, 10.8% and 17.3% at low, mid and high doses, respectively) spleen (23.3% at high dose), pancreas (26.6% and 71.3% at mid and high doses, respectively), testes (21.6% at high dose), lung (24.2% at high dose), heart (11.6% at high dose), stomach (29.3%, 67.3% and 103% at low, mid and high doses, respectively) and cecum/colon (12%, 32.7% and 75% at low, mid and high doses, respectively) of the treatment group males were higher than that of controls. In females, the relative weights of liver (19% at high dose), spleen (48.4% at high dose), pancreas (33.3% at high dose), uterus (55% at high dose), lung (19.8% at high dose), heart (9.5% at high dose), adrenal (44.8% and 43.8% at mid and high doses, respectively), stomach (60.5% and 129.4% at mid and high doses, respectively) and cecum/colon (28.8% at high dose) of animals receiving RU-0211 were higher than that of controls.

Histopathology:

Nonneoplastic: Male rats treated with RU-0211 for 104 weeks had significantly higher incidences of hyperplasia, hyperkeratosis, edema and keratinic cyst in the nonglandular stomach, when compared with the controls. High dose males had necrosis of the glandular stomach and ulceration and necrosis of the cecum. Male animals receiving 100 and 400 µg/kg doses had mineralization of the vascular wall of the testes. Treatment group females had higher incidences of hyperplasia, hyperkeratosis and edema, and high dose females had higher incidences of keratinic cyst in the nonglandular stomach. Increased hyperplasia was also observed in the glandular stomach of females receiving 100 and 400 µg/kg doses.

The non-neoplastic changes observed in male and female rats are shown in the Table below.

Organ/Lesion	Control	20 µg/kg	100 µg/kg	400 µg/kg
MALES				
Lung				
-pleuritis	0/65	0/65	0/65	2/65
Heart				
-vessel mineralization	0/65	2/65	1/65	5/65
Spleen				
-extramedullary hematopoiesis	0/65	4/65	2/65	6/65
Kidney				
-suppurative pyelonephritis	0/65	1/65	2/65	2/65
Stomach, nonglandular				
-hyperplasia	3/65	51/65	60/65	57/65
-hyperkeratosis	4/65	17/65	21/65	46/65
-edema	2/65	14/65	23/65	43/65
-keratinic cyst	1/65	2/65	7/65	11/65
Stomach, glandular				
-necrosis	0/65	1/65	0/65	3/65
Cecum				
-necrosis	0/65	0/65	1/65	2/65
-ulceration	1/65	0/65	1/65	4/65
Testes				
-mineralization of vascular wall	0/55	0/65	2/65	4/65
FEMALES				
Adrenal cortex				
-necrosis	0/65	1/65	0/65	2/65
Stomach, nonglandular				
-hyperplasia	4/65	44/65	60/65	58/65
-hyperkeratosis	3/65	19/65	31/65	27/65
-edema	4/65	16/65	26/65	33/65
-keratinic cyst	0/65	0/65	1/65	10/65
Stomach, glandular				
-hyperplasia	1/65	1/65	3/65	3/65

Neoplastic:

Male rats: In male rats, a positive trend for squamous cell papilloma in nonglandular stomach [control, 1/65 (1.5%); low dose, 1/65 (1.5%), mid dose, 5/65 (7.7%), high dose, 6/64 (9.4%); p=0.0135, trend test] was observed. A positive trend was also observed for the combined incidences of squamous cell papilloma and carcinoma in male rats [control, 2/65 (3.1%); low dose, 1/65 (1.5%), mid dose, 6/65 (9.2%), high dose, 7/64 (10.9%); p=0.0156, sponsor; trend test]. The incidences of histiocytic sarcoma [control, 6/65 (9.2%); low dose, 1/65 (1.5%), mid dose, 4/65 (6.2%), high dose, 10/65 (15.4%); p=0.0015, sponsor, p=0.0091, CDER statistician; trend test] and benign interstitial cell tumor of the testes [control, 2/65 (3.1%); low dose, 4/65 (6.2%), mid dose, 1/65 (1.5%), high dose, 10/65 (15.4%); p=0.0002, sponsor; p=0.0006, CDER statistician; trend test] were significantly higher in high dose male rats.

Female rats: A positive trend was observed for the incidences of hepatocellular adenoma [control, 0/65 (0%); low dose, 0/65 (0%), mid dose, 1/65 (1.5%), high dose, 5/65 (7.7%); p=0.0012, sponsor; p=0.0031, CDER statistical; trend test] in female rats.

The tumor incidences in male and female rats are shown in the Table below.

Organ/tumor	Control	20 µg/kg	100 µg/kg	400 µg/kg	P value (Trend test)
MALES					
Stomach, Nonglandular					
-Squamous cell papilloma	1/65 (1.5%)	1/65 (1.5%)	5/65 (7.7%)	6/64 (9.4%); (p=0.035, pair wise testing)	0.0135
-Squamous cell carcinoma	1/65 (1.5%)	0/65 (0%)	1/65 (1.5%)	1/64 (1.5%)	--
-Squamous cell papilloma + carcinoma	2/65 (3.1%)	1/65 (1.5%)	6/65 ((9.2%)	7/64 (10.9%); (p=0.0518, pair wise testing)	0.0156
Hemato neoplasia, Histiocytic sarcoma	6/65 (9.2%)	1/65 (1.5%)	4/65 (6.2%)	10/65 (15.4%); (p=.0697, pair wise testing)	0.0015
Testes					
-Beign interstitial cell tumor	2/65 (3.1%)	4/65 (6.2%)	1/65 (1.5%)	10/65 (15.4%); (p=0.0035, pair wise testing)	0.0002
FEMALES					
Liver					
-Hepatocellular adenoma	0/65 (0%)	0/65 (0%)	1/65 (1.5%)	5/65 (7.7%)	0.0012
-Hepatocellular carcinoma	0/65 (0%)	0/65 (0%)	0/65 (0%)	0/65 (0%)	

Toxicokinetics: The plasma RU-0211 concentrations male and female rats treated with a 20 µg/kg dose were below the lower limit of quantification (— pg/ml). The mean maximum plasma concentrations of RU-0211 were reached at 1 hr after dosing, and were below the quantification limit at 24 hr after dosing. The mean C_{max} and AUC values increased with increasing dose from 100 to 400 µg/kg, and no apparent differences in the exposure levels were observed between males and females. Toxicokinetic parameters for RU-0211 in male and female rats are summarized in the Table below.

Summary of Toxicokinetic Data

Sex/Group µg/kg/day	Male 2 20	Male 3 100	Male 4 400	Female 2 20	Female 3 100	Female 4 400
Cmax (pg/mL)						
Day 1	NC	94.0	134	NC	148	303
Week 26	NC	66.6	268	NC	183	513
Tmax (hr)						
Day 1	NC	3.50	0.833	NC	1.50	2.67
Week 26	NC	1.00	3.00	NC	1.00	0.833
$AUC_{0\text{-}24hr}$ (pg·hr/mL)						
Day 1	NC	450	464	NC	709	2170
Week 26	NC	207	2610	NC	216	2750

NC not calculated
N 1 for 100 ug/kg/day males at Week 26; otherwise, N = 2 or 3.

Summary of individual study findings:

In the 2-year oral gavage carcinogenicity study with RU-0211 in rats, the drug was administered at 20, 100 and 400 μg/kg doses. The doses were selected on the basis of the MTD from a 26-week oral toxicity study in rats, and concurred by the CDER Executive CAC Committee. Treatment with RU-0211 had no adverse effects on the survival of male and female rats. The mean body weights of the mid and high dose males were slightly lower than that of controls in most part of the dosing period.

Male rats treated with RU-0211 had significantly higher incidences of hyperplasia, hyperkeratosis, edema and keratinic cyst in the nonglandular stomach, when compared with the controls. Treatment group females had higher incidences of hyperplasia, hyperkeratosis and edema, and high dose females had higher incidences of keratinic cyst in the nonglandular stomach. Increased hyperplasia was also observed in the glandular stomach of females receiving 100 and 400 μg/kg doses.

In male rats, a positive trend for the incidences of squamous cell papilloma in the nonglandular stomach [control, 1/65 (1.5%); low dose, 1/65 (1.5%), mid dose, 5/65 (7.7%), high dose, 6/64 (9.4%); p=0.0135, trend test] was observed. A positive trend was also observed for the combined incidences of squamous cell papilloma and carcinoma in male rats [control, 2/65 (3.1%); low dose, 1/65 (1.5%), mid dose, 6/65 (9.2%), high dose, 7/64 (10.9%); p=0.0156, trend test]. However, according to the CDER standard, for statistical significance, the p values for common tumors should be <0.005. Thus, according to the CDER criteria, the incidences of non-glandular stomach squamous cell papilloma + carcinoma in male rats were not significant. There was a significant positive trend for the incidences of histiocytic sarcoma [control, 6/65 (9.2%); low dose, 1/65 (1.5%), mid dose, 4/65 (6.2%), high dose, 10/65 (15.4%); p=0.0015, sponsor; p=0.0091, CDER statistician; trend test] and benign interstitial cell tumor of the testes [control, 2/65 (3.1%); low dose, 4/65 (6.2%), mid dose, 1/65 (1.5%), high dose, 10/65 (15.4%); p=0.0002, sponsor; p=0.0006, CDER statistician; trend test] in male rats. Although, there was positive trend for the incidences of histiocytic sarcoma in male rats, the incidences of this tumor at any dose were not significantly different from the control incidences.

The incidences of squamous cell papilloma and combined incidences of papilloma and carcinoma in the nonglandular stomach of male rats were higher than historical control incidences (squamous cell papilloma- 0% – 1.5%, mean 0.66%; papilloma+carcinoma – 0% to 3%, mean 1.42%) in this strain of rat from the conducting laboratory. The incidences of histiocytic sarcoma and interstitial cell tumor of the testes in male rats were also higher than the conducting laboratory's historical control incidences in this strain of rat (histiocytic sarcoma – 0% to 4.3%, mean 0.87%; interstitial cell tumor of testes – 0% to 8.3%, mean 3.2%). The incidences of testicular interstitial cell tumors in male rats were also higher than the background incidences in this strain (1.45%-7.14%; mean, 2.35%; Charles River Laboratories).

In female rats, a significant positive trend for the incidences of hepatocellular adenoma [control, 0/65 (0%); low dose, 0/65 (0%), mid dose, 1/65 (1.5%), high dose, 5/65 (7.7%); p=0.0012, sponsor; p=0.0031, CDER statistician; trend test] was observed. The incidences of hepatocellular adenoma in female rats were higher than the historical control incidences for this strain of rat from the conducting laboratory (0% to 1.5%, mean 0.8%). The incidences of hepatocellular adenoma in

female rats at the high dose were higher than the mean background incidences in this strain (2.02%; range, 0.77%-13.3%; Charles River Laboratories).

Adequacy of the carcinogenicity study and appropriateness of the test model: The dose selection for the 2-year rat carcinogenicity study was adequate, and was concurred by the CDER Executive CAC Committee. The conduct of the study was appropriate and animal survival was sufficient for an adequate assessment of tumorigenic potential.

Evaluation of tumor findings:
In male rats, a positive trend for the incidences of squamous cell papilloma in the nonglandular stomach was observed. A positive trend was also observed for the combined incidences of squamous cell papilloma and carcinoma in male rats. However, according to CDER standard, the incidences of non-glandular stomach squamous cell papilloma + carcinoma in male rats were not significant ($p>0.005$). There was a significant positive trend for the incidences of histiocytic sarcoma and benign interstitial cell tumor of the testes in male rats.
The incidences of squamous cell papilloma and combined incidences of papilloma and carcinoma in the nonglandular stomach of male rats were higher than historical control incidences in this strain of rat from the conducting laboratory. The incidences of histiocytic sarcoma and interstitial cell tumor of the testes in male rats were also higher than the conducting laboratory's historical control incidences.
For female rats, a significant positive trend for the incidences of hepatocellular adenoma was observed. The incidences of hepatocellular adenoma in female rats were higher than the historical control incidences for this tumor from the conducting laboratory.

Carcinogenicity conclusions:

In the 2-year oral (gavage) carcinogenicity study with RU-0211 in rats, groups of animals received 0, 20, 100 and 400 µg/kg/day doses of the drug. In male rats, a significant positive trend was observed for the incidences of histiocytic sarcoma and benign interstitial cell tumor of the testes. In female rats, a significant positive trend was observed for the incidences of hepatocellular adenoma.

Recommendations for further analysis: None
Labeling recommendations: The tumor incidences in male and female rats should be included in the labeling.

2.6.6.6 Reproductive and developmental toxicology

Study title: Effects of RU-0211 on Fertility and Early Development of Embryo in Rats by Oral Administration.

Study No. 100116

Conducting laboratory (and location if not the Sponsor):

Dates of study initiation & completion: March 14, 1997 & June 19, 1998

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: RU-0211, Lot # 6.

Protocol reviewed by the Division? No

METHODS:

Species: Male (298-344 g)

Female (217-267 g)

: CD Sprague-Dawley rats

Doses employed and Route of Administration: Four groups of male and female animals received RU-0211 at oral doses of 0. 0.04, 0.2 or 1.0 mg/kg/day (5 ml/kg). The drug, dissolved in distilled water containing Polysorbate 80 (1% w/v), was administered by oral gavage. The doses were selected on the basis of a 4-week oral toxicity study with RU-0211 in rats (Study # 500116) in which male and female animals received 0.04, 0.2 and 1.0 mg/kg/day of the drug. Drug related toxicities were observed at 1.0 mg/kg/day. So the sponsor selected 1.0 mg/kg as the high dose for the Segment I fertility study and the mid- and the low doses were selected as 0.2 and 0.04 mg/kg/day respectively.

Study Designs: The male animals received RU-0211 for a total of 63-66 days, beginning 28 days prior to mating and continued for 35 days after the first mating. The females received the drug for a maximum of 36 days, beginning 14 days prior to mating and continued through Day 7 of gestation.

Number of animals/sex/dosing group: 20 animals/sex/group

Parameters and endpoints evaluated: The animals were observed for general signs and mortalities twice a day. The body weights of the males were measured twice a week and the food consumption was measured once a week. The body weights and food consumption of the females were measured twice a

week during the premating and the mating periods and once a day during the gestation period. Mating was confirmed by the presence of sperms in the vaginal smears. Male animals were sacrificed on the day following the last dosing and necropsies performed. The testes and epididymides were weighed and fixed in Bouin's solution. Organs and tissues showing abnormalities at necropsy were fixed in buffered formalin. Sperm motility and sperm viability was examined on all animals. The female animals were sacrificed on Day 13 of pregnancy and necropsies performed. The number of corpora lutea and implantation were counted and the ovaries and the uteri were fixed in buffered formalin.

Statistical evaluation: The homogeneity of variance of all parameters was tested by the Bartlett's method. After the homogeneity of variance was confirmed, one way analysis of variance (ANOVA) was used to determine the significance, and the data was further analyzed by Dunnett's method. Copulation and fertility indexes were analyzed by χ^2 test.

RESULTS:

Clinical signs:

Males: Salivation was observed in all groups of animals receiving RU-0211 (salivation was noted from Days 32, 26 and 9 in the low, mid- and high dose groups respectively) and it lasted for about 30 minutes after dosing. The number of animals showing salivation was dose dependent (11 in the 0.04 mg/kg group and 20 each in 0.2 and 1.0 mg/kg group). Loose stool and abdominal distension, beginning from Day 9-10, was observed in animals receiving the high dose (all animals had loose stool and 8 (of 20) animals had abdominal distension).

Females: Females receiving 0.2 mg/kg and 1.0 mg/kg doses had salivation beginning from Day 16 and Day 8 of dosing respectively. The salivation lasted for about 30 minutes after dosing, and continued until the last day of dosing. The maximum incidences of salivation per day were dose dependent. The animals in the 1.0 mg/kg group had loose stool (all animals had loose stool from Day 9 of dosing to Day 12 of pregnancy) and abdominal distention (7 of 20 animals had abdominal distention from Day 12 of dosing to Day 13 of pregnancy).

Mortality: There were no deaths in any groups.

Body weight and food consumption: The initial mean body weight of the control males and females were 319.9±10.4 and 240.9±13.0 g respectively. The final body weights of the males and females were 509.2±26.2 and 323.9±21.2 g respectively. The high dose males had slightly lower than control body weights from Day 8 to Day 64 of dosing (6.7%-9.9%). The body weight gains of the females receiving 0.2 and 1.0 mg/kg doses were lower than control on Day 7 (22.2% and 25.5% respectively), 8 (20.1% and 29.0% respectively) and 13 (22.4% and 16.6% respectively) of pregnancy.

The mean food consumption of the control male and female animals at the beginning of the dosing period were 22.2±3.5 g and 15.9±3.4 g/day respectively. The food consumption of the males receiving the low and the medium dose was higher than control on Day 23 of dosing (15.7% at both doses), while the high dose males had lower food consumption on Day 2 of dosing (36%). The high dose females had decreased food consumption on days 2 and 5 of dosing (45.3% and 22.3% respectively).

Toxicokinetics in parental animals: Not done.

Fertility in Males: There were no differences in sperm counts and sperm motility between the control and the treatment groups. There were no differences in the histopathological findings between the control and the treatment groups.

Fertility and Early Embryonic Development in Females: No significant differences in copulation and fertilization ratio was observed between the control and the treatment groups (infertility was observed in 1 (of 20) animal in the 0.04 mg/kg group and 3 (of 20) animals each in 0.2 and 1.0 mg/kg groups). There were no significant differences in the number of corpora lutea, implantation index, and pre-implantation losses between the control and the treatment groups. The number of implantation sites (control, 16.6±2.2 and 1.0 mg/kg, 14.4±3.1) and live embryos (control, 15.8±2.4 and 1.0 mg/kg, 12.9±2.8) were significantly lower than control in the 1.0 mg/kg group. The number of dead or resorbed embryos in the 1.0 mg/kg group was also lower but not statistically significant. The changes were not observed in untreated females mated with treated males; thus the changes observed were due to the effects of the prostaglandin analog on the female animals. The reproductive performance and early embryonic and fetal development data for rats receiving RU-0211 is shown in the sponsor-provided tables below.

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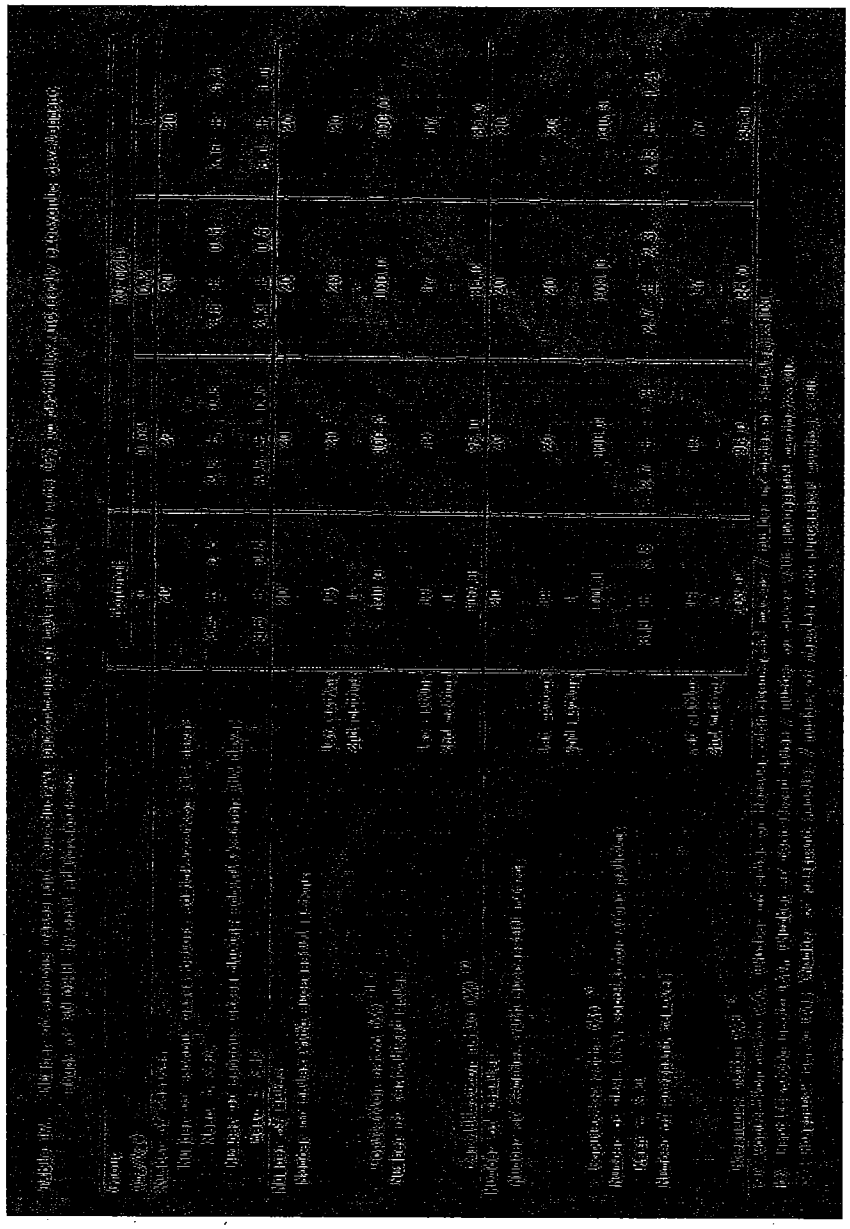
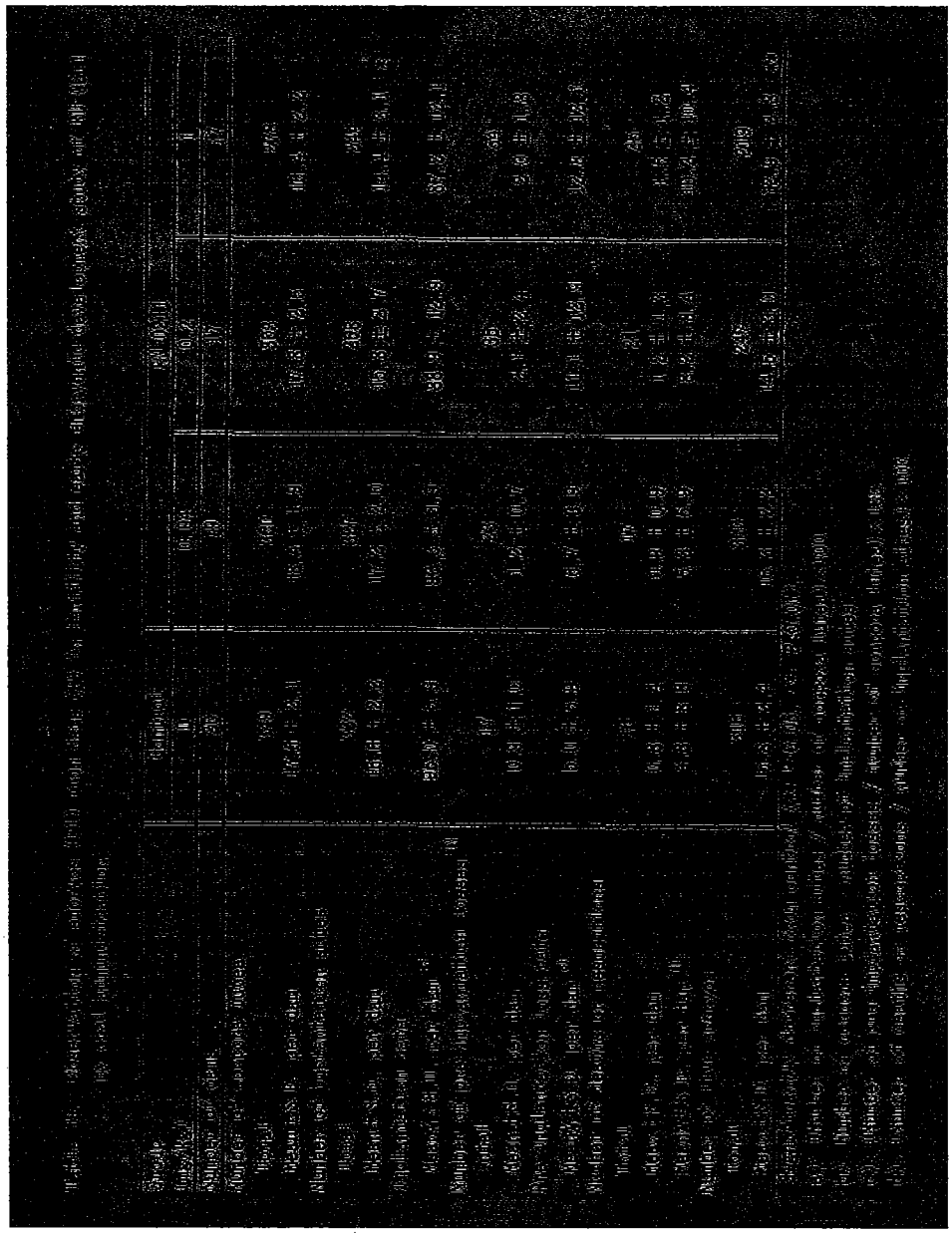




In the Segment I fertility and early embryonic development study in rats, male and female animals received 0.04, 0.2 and 1.0 mg/kg doses of RU-0211. RU-0211 had no effects on the fertility of the male and female animals. The number of implantation sites and live embryos were lower than control in the high dose group. The effects of RU-0211 on implantation are attributable to its effects on the female animals, since no abnormalities were found after mating untreated females with treated males.

Study Title: Rat Developmental Toxicity Study – Evaluation of Oral Capsule RU-0211 Administration (Segment II Teratogenicity Study with RU-0211 in Rats by the Oral Route) (Study # RTU/SR01-062)

Key study findings: In the oral Segment II teratogenicity study with RU-0211 in rats, pregnant females received 0. 0.02, 0.2 and 2.0 mg/kg/day doses from Gestation Day 6 through Gestation Day 17. Fetal malformations such as situs inversus (lateral transposition of the abdomen and thorax), three abnormal lobes in the lung, ventricular septal defect in the heart, cleft palate and hydronephrosis observed in animals receiving the 2 mg/kg/day dose. However, this dose was toxic to the mothers. No adverse effects on the fetuses were observed at doses up to 0.2 mg/kg/day.

Study Report No # RTU/SR01-062

Volume # and page #: Volume #3, Page # 1-237.

Conducting laboratory and location: —

Dates of study initiation and completion: November 22, 2000 and July 24, 2001.

GLP compliance: Yes

QA Report Yes (X) No ( )

Drug, Lot #, radiolabel, and % purity: RU-0211 was dissolved in medium chain fatty acid triglyceride (MCT) at 5 different concentrations: 0.5 mg/g (Batch # 001121-2); 5.0 mg/g (Batch # 001121-3); 50 mg/g (Batch # 001121-4) and encapsulated in microcapsules. The purity of each batch ranges from — The capsules were prepared daily.

Methods:

Species/strain: CD (SD)IGS BR VAF/Plus® rats.

Doses employed: Four groups of pregnant female rats received oral doses of 0, 0.02, 0.2 and 2.0 mg/kg/day of RU-0211 from Gestation Day 6 through Gestation Day17. The doses were selected on the basis of a dose range-finding study in which 0, 0.016, 0.08, 0.4 and 2.0 mg/kg/day doses were administered to pregnant female rats from Gestation Day 6 through Gestation Day 17. Treatment-related adverse effects such as loose stools, decreased body weights, decreased food consumption and thickening of the mucosa of the non-glandular stomachs were observed in the high dose animals. Based on these findings, the sponsor selected 2.0 mg/kg/day as the high dose for this Segment II teratogenicity study in rats. The mid and the low doses were 10 and 100 times lower than the high dose, respectively.

Route of administration: Oral

Study design: Four groups of pregnant rats received 0, 0.02, 0.2 and 2.0 mg/kg/day doses of RU-0211 from gestation day 6 through gestation day 17. Each group of animal received the appropriate dosing formulation, administered as oral capsules.

Number/sex/group: 25 animals/group.

Parameters and endpoints evaluated: The animals were observed twice daily for clinical signs and mortality. The body weights and food consumption were recorded on gestation days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20. On day 20 of gestation, all surviving dams were sacrificed and examined for gross abnormalities. The uteri were weighed and examined for implantation sites and an evaluation was made to determine the number of early- or late-resorptions, dead fetuses and apparently normal developing fetuses. The ovaries were examined for the number of corpora lutea. Each fetus was sexed, weighed and examined for external abnormalities. Approximately one-half of the fetuses from each litter were examined for visceral abnormalities by the Wilson technique. The remaining fetuses were examined for skeletal abnormalities by the Alizarin red S staining method. Developmental deviations were judged as variations and malformations.

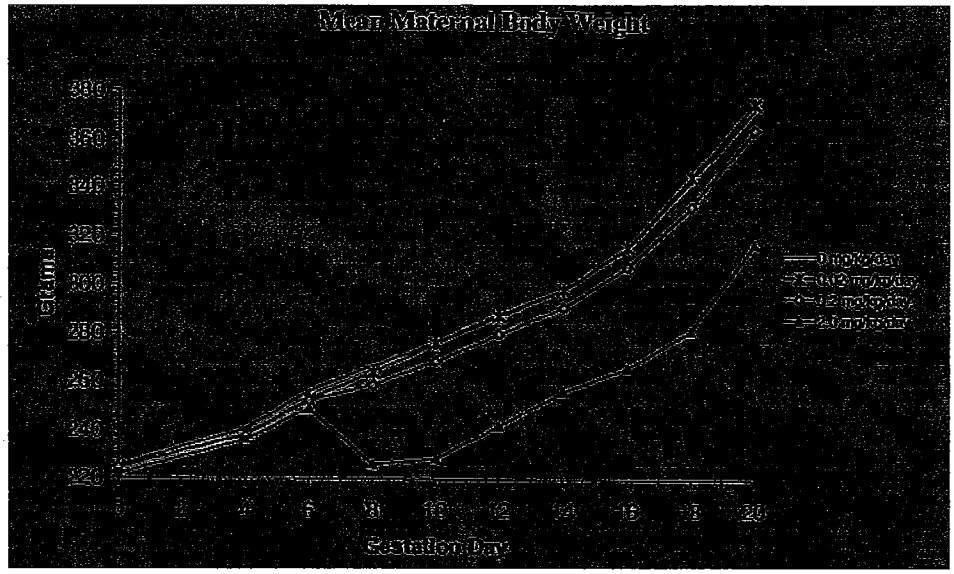
Results:

In-life observations:

Mortality: A total of six animals died and/or was sacrificed moribund due to deteriorating health conditions. Three animals, one each in the control, 0.02 mg/kg/day and 2.0 mg/kg/day group died due to complications of capsule dosing. Another control animal was found dead on gestation day 17; the cause of the death was not known. Two high dose animals were sacrificed *in extremis* on gestation days 12 and 17 due to treatment-related significant decreases in the body weights.

Clinical signs: Treatment related clinical signs were limited to the high dose group and included soft stools (25 of 25 animals), yellow staining of furs (15 of 25 animals), thin appearance (5 of 25 animals), red crust on the nose (5 of 25 animals) and chromodacryorrhea (2 of 25 animals).

Body weight: The mean body weights of the control animals at the beginning (day 6 of gestation) and end (day 18 of gestation) of dosing were 256.5±13.6 and 343.2±23.4 g, respectively. Beginning from gestation day 8, the high dose animals had lower body weights throughout the dosing period, as compared with the controls (15.3%, 14.1% and 17.9% decreases on gestation days 8, 14 and 18, respectively). The body weight gain of the high dose animals was 62% lower than that of controls during this period (86.23 g gain in controls vs. 32.95 g in the high dose group). The mean body weights of different groups of animals during gestation day 0 to gestation day 20 are shown in the sponsor's Figure below.



Food consumption: The mean food consumption of the control animals before beginning of dosing (gestation days 4-6) and at the end of dosing (gestation days 16-18) was 28.0 ± 2.4 and 31.5 ± 2.9 g/animal/day. The food consumption of the high dose animals was lower than that of controls throughout the dosing period (69.9%, 9.3% and 17.1% decreases on gestation days 6-8, 12-14 and 16-18, respectively. On gestation days 18-20, the high dose animals had 42.9% higher food consumption as compared with the controls.

Terminal and macroscopic evaluations:

The mean gravid uterus weight of the high dose animals was lower (38.4%) than that of controls; no changes were observed in any other groups. The number of dams with viable fetuses was lower in the high dose group as compared with controls (75% vs. 100%). No treatment-related changes in the number of corpora lutea, implantation sites, and pre- and post- implantation losses were observed in any group. Animals receiving the 2 mg/kg/day dose had increased incidences of early resorptions. Six of 23 pregnant females in this group had early resorptions, and 5 of these 6 animals had complete litter resorptions. Treatment with RU-0211 had no effect on the number of live fetuses, sex ratio and mean fetal weights. The mean ovarian, uterine and litter data are summarized in the Table below

	0 mg/kg/day	0.02 mg/kg/day	0.2 mg/kg/day	2.0 mg/kg/day
Number mated	25	25	25	25
Number pregnant	24 (96%)	25 (100%)	25 (100%)	25 (100%)
Number aborted	0	0	0	0
Number dead/sacrificed	2 (8%)	1 (4%)	0	3 (12%)
Dams with viable fetuses	22 (100%)	24 (100%)	25 (100%)	15 (75%)
Corpora lutea (mean ± S.D)	15.9 ± 1.9	15.6 ± 2.3	15.8 ± 2.7	15.1 ± 2.1
Implantation sites (mean ± S.D)	13.9 ± 1.6	14.2 ± 1.7	13.8 ± 2.0	13.2 ± 1.6
Pre-implantation loss (%mean ± S.D)	11.7 ± 9.9	8.3 ± 9.3	12.0 ± 9.5	11.4 ± 10.4
Post-implantation loss (%mean ± S.D)	4.8± 9.3	3.7 ± 5.9	6.1 ± 11.8	32.1 ± 44.9
Live fetuses (mean ± S.D)	13.2 ± 1.6	13.7 ± 1.9	12.9 ± 2.3	9.1 ± 6.1
Sex ratio (M : F)	50 : 50	50 : 50	52 : 48	56 : 44
Resorptions, total (mean ± S.D)	0.7 ± 1.6	0.5 ± 0.8	0.9 ± 1.7	1.3 ± 3.3
Early resorptions (mean ± S.D)	0.7 ± 1.6	0.5 ± 0.8	0.9 ± 1.7	1.3 ± 3.3
Late resorptions (mean ± S.D)	0.1 ± 0.2	0 ± 0	0 ± 0	0.1 ± 0.3
Dead fetuses	0	0	0	0
Fetal weights (mean ± S.D)	3.59 ± 0.23	3.43 ± 0.29	3.54 ± 0.27	3.36 ± 0.42

The high dose group had higher incidences of fetal soft tissue malformations. A total of 5 fetuses from 3 litters (dams B34611, B34612 and B34668) had multiple soft tissue malformations that included situs inversus (lateral transposition of the abdomen and thorax, observed in 2 litters), three abnormal lobes in the lung, and ventricular septal defect in the heart. Three fetuses from dam B34612 had cleft palate and one of these 3 fetuses had hydronephrosis. No treatment-related effects on skeletal variations or malformations were observed in any group. Fetal soft tissue and skeletal malformations are summarized in the Table below.

	0 mg/kg/day	0.02 mg/kg/day	0.2 mg/kg/day	2.0 mg/kg/day
Litters evaluated	22	24	25	14
Fetuses evaluated	145	163	161	88
Fetal Soft Tissue Variations				
Dilatation of lateral ventricles				
- Fetal incidence	1 (0.7%)	2 (1.2%)	0 (0.0%)	3 (3.4%) *
- Litter incidence	1 (4.5%)	2 (8.3%)	0 (0.0%)	2 (14%)
Increased renal pelvic cavitation				
- Fetal incidence	8 (5.5%)	11(6.7%)	7 (4.3%)	2 (2.3%)
- Litter incidence	4 (18%)	5 (21%)	5 (20%)	2 (14%)
Dilated ureter(s)				
- Fetal incidence	8 (5.5%)	9 (5.5%)	3 (1.9%)	4 (4.5%)
- Litter incidence	5 (23%)	6 (25%)	3 (12%)	2 (14%)
Fetal Soft Tissue Malformations				
Situs inversus				
- Fetal incidence	0 (0.0%)	2 (1.2%)	0 (0.0%)	2 (2.3%)
- Litter incidence	0 (0.0%)	2 (8.3%)	0 (0.0%)	2 (14%)
Cleft palate				
- Fetal incidence	0 (0.0%)	0 (0.0%)	1 (0.6%)	3 (3.4%)
- Litter incidence	0 (0.0%)	0 (0.0%)	1 (4.0%)	1 (7.1%)
Hydronephrosis				
- Fetal incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)
- Litter incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)
Total soft tissue malformations				
- Fetal incidence	0 (0.0%)	3 (1.8%)	1 (0.6%)	5 (5.7%) **
- Litter incidence	0 (0.0%)	3 (13%)	1 (4%)	3 (21%)
Fetal Skeletal Variations				
Litters Evaluated	22	24	25	15
Fetuses Evaluated	145	165	162	93
Incomplete/unossified hyoid body				
- Fetal incidence	24 (17%)	19 (12%)	22 (14%)	4 (4.3%) **
- Litter incidence	12 (55%)	9 (38%)	10 (40%)	2 (13%) *
Incomplete ossification of the skull				
- Fetal incidence	12 (8.3%)	15 (9.1%)	6 (3.7%)	2 (2.2%) *
- Litter incidence	6 (27%)	8 (33%)	3 (12%)	2 (13%)

Less than four caudal vertebrae ossified - Fetal incidence - Litter incidence	31 (21%) 10 (45%)	36 (22%) 14 (58%)	11 (6.8%) 8 (32%)	27 (29%)** 9 (60%)
Incomplete ossification of vertebral arch - Fetal incidence - Litter incidence	14 (9.7%) 10 (45%)	22 (13%) 12 (50%)	6 (3.7%) * 3 (12%) *	1 (1.1%) * 1 (6.7%) *
5^{th} sternebra unossified - Fetal incidence - Litter incidence	46 (32%) 15 (68%)	48 (29%) 17 (71%)	34 (21%) * 15 (60%)	27 (29) * 8 (53%)
$5^{th}/6^{th}$ sternebra bipartite - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)	1 (0.6%) 1 (4%)	4 (4.3%) 3 (20%)

*, $p<0.05$; **, $p<0.01$

Summary:

In the segment II teratogenicity study with RU-0211 capsules in rats, groups of pregnant females received 0, 0.02, 0.2 and 2.0 mg/kg/day doses of the drug from Gestation Day 6 through Gestation Day 17. Animals receiving the high dose had fetuses with higher incidences of fetal soft tissue malformations that included situs inversus (lateral transposition of the abdomen and thorax), three abnormal lobes in the lung, ventricular septal defect in the heart, cleft palate and hydronephrosis. These malformations are within the supplier's range of spontaneous incidences in this species. Moreover, the effects were observed at a dose that was maternotoxic (there were mortalities and decreased body weights at this dose). No fetal abnormalities were observed at 0.02 and 0.2 mg/kg/day doses.

Study Title: Developmental Toxicity Study with Oral Administration of RU-0211 in Rabbits (Segment II Teratogenicity Study).

Key study findings: In the segment II teratogenicity study with RU-0211 in rabbits, groups of pregnant females received 0, 0.01, 0.03 and 0.10 mg/kg/day doses of the drug from Gestation Day 7 through Gestation Day 20. RU-0211 was not teratogenic in rabbits at oral doses up to 0.10 mg/kg/day.

Study No # SPI/SR03-039

Volume # and page #: Volume #12, Page # 1-230.

Conducting laboratory and location:

Dates of study initiation and completion: September 04, 2002 and April 10, 2003.

GLP compliance: Yes

QA Report Yes (X) No ( )

Drug, Lot #, radiolabel, and % purity: RU-0211; Lot Nos. YY020902, YY020902-3, and YY020902-4.

Methods:

Species/strain: New Zealand White rabbits.

Doses employed: Four groups of pregnant rabbits received oral doses of 0, 0.01, 0.03 and 0.10 mg/kg/day of RU-0211 from Gestation Day 7 through Gestation Day 20. The doses were selected on the basis of a dose range-finding study (Study # 7142-103) in which 0, 0.003, 0.03 and 0.1 mg/kg/day doses were administered to pregnant female rabbits from Gestation Day 7 through Gestation Day 20. There were decreased food consumption and decreased body weights (7% on gestation day 19) of the high dose animals during the dosing period. One (of 5) animal receiving the high dose had an abortion on gestation day 27. Based on these findings, the sponsor selected 0.1 mg/kg/day as the high dose for this Segment II teratogenicity study in rabbits.

Route of administration: Oral

Study design: Four groups of pregnant rabbits received 0, 0.01, 0.03 and 0.1 mg/kg/day doses of RU-0211 from gestation day 7 through gestation day 20. The control animals received the vehicle (medium chain triglyceride, MCT). The concentrations of RU-0211in MCT for groups 1, 2, 3 and 4 were 0, 0.5, 1.5 and 5.0 mg/g, respectively. Each group of animal received the respective dosing formulation, administered as oral capsules. On gestation day 29, all females were sacrificed and examined for gross abnormalities. The fetuses were collected immediately by cesarean section and examined.

Number/sex/group: 20 animals/group.

Parameters and endpoints evaluated: The animals were observed twice daily for clinical signs and mortality. The body weights and food consumption were recorded on gestation days 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27 and 29. Animals that died prior to the scheduled sacrifice were examined macroscopically for gross abnormalities of the thoracic and abdominal cavities, and pelvic viscera. The uterus and ovaries were examined for implantations and corpora lutea, respectively. The uterus was examined for early or late resorbing fetuses, dead fetuses, or apparently normally developing fetuses. On day 29 of gestation, all surviving females were sacrificed and examined for gross abnormalities. The uteri were weighed and examined for implantation sites and an evaluation was made to determine the number of early- or late-resorptions, dead fetuses and apparently normal developing fetuses. The ovaries were examined for the number of corpora lutea. Each fetus was weighed and examined for external abnormalities. The fetuses were examined for soft tissue abnormalities, during which the sex was determined. Skeletal abnormalities of the fetuses were examined by the Alizarin red S staining method and included examinations of the skull, vertebral column, rib cage, pectoral and pelvic girdles and long bones and extremities. Developmental deviations were judged as variations and malformations.

Results:

Mortality: There were two unscheduled deaths. One female rabbit receiving the 0.01 mg/kg/day dose (F60259) died on GD 17, and one animal receiving the 0.03 mg/kg/day dose (F60281) delivered early on the day of scheduled sacrifice, and was subsequently euthanized. Necropsy findings of rabbit # F60259 included reddened trachea, fluid-filled thoracic cavity, failure of lungs to collapse and perforation of the diaphragmatic lobe of the lungs, indicating that a gavage error was the cause of death of this animal.

Clinical signs: No treatment related clinical signs were observed in any group.

Body weight: The mean body weights of the control animals before beginning of dosing (GD 0) and at the end of dosing (GD21) were 3468.4 ±179.6 and 4064.4 ±298.5 g, respectively. There were no treatment-related changes in the body weights of animals in any group. The body weight gain of the high dose animals was slightly lower than that of controls during the dosing period (8% during GD 4-21).

Food consumption: The mean food consumptions of the control animals before initiation of dosing (GD 4-7) and at the end of dosing (GD21-29) were 161.2 ± 30 and 141.8 ± 46.8 g/kg/day, respectively. The food consumption of the high dose animals was lower than that of controls throughout the dosing period (8%, 28% and 11% decreases on gestation days 9-11, 13-15 and 15-18, respectively.

In-life observations: The pregnancy rates were 85%, 90%, 95% and 90% in the control, low, mid and high dose groups, respectively. There were no abortions in any group. One animal receiving the 0.03 mg/kg/day dose delivered early on the scheduled day of necropsy (GD 29), and was subsequently euthanized. Two animals in the vehicle control group and two animals in the high dose groups had litters with no viable fetuses. Post-implantation loss and the number of viable fetuses were not affected by treatment with RU-0211. The pregnancy and litter data for different groups are summarized in the Table below.

Parameter	0 mg/kg/day	0.01 mg/kg/day	0.03 mg/kg/day	0.10 mg/kg/day
Number mated	20	20	20	20
Number pregnant	17 (85%)	18 (90%)	19 (95%)	18 (90%)
Delivered early	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Pregnant at Cesarean section	17	1 7	19	18
Dams with viable fetuses	15 (88%)	17 (100%)	19 (100%)	16 (89%)
Dams with no viable fetuses	2 (12%)	0 (0%)	0 (0%)	2 (11%)

Terminal and macroscopic evaluations:

Dams: There were no treatment-related gross abnormalities at necropsy in any group. The mean gravid uterine weights in all groups were similar. Treatment of the female rabbits with RU-0211 during GD 7 through 20 had no effect on the number of implantation sites, early or late resorptions and the number of live or dead fetuses. The sex ratio and fetal weights were also not affected by RU-0211. The cesarean section data for dams from different treatment groups are summarized in the Table below.

Parameter	0 mg/kg/day	0.01 mg/kg/day	0.03 mg/kg/day	0.10 mg/kg/day
Number mated	20	20	20	20
Number pregnant	17 (85%)	18 (90%)	19 (95%)	18 (90%)
Corpora lutea (mean ± S.D)	9.2 ± 1.7	10.4 ± 2.3	9.6 ± 1.7	8.8 ± 2.6
Implantation sites (mean ± S.D)	7.7 ± 2.6	8.6 ± 2.0	8.3 ± 2.4	7.2 ± 3.0
Pre-implantation loss (mean ±S.D)	18.5 ± 19.1	16.9 ± 15.6	12.6 ± 20.7	18.9 ± 19.9
Resorptions, total (mean ± S.D)	1.2 ± 2.3	0.6 ± 0.9	0.6 ± 1.3	0.7 ± 1.1
Early resorptions (mean ± S.D)	0.9 ± 2.3	0.4 ± 0.6	0.5 ± 1.3	0.5 ± 0.9
Late resorptions (mean ± S.D)	0.2 ± 0.4	0.2 ± 0.6	0.1 ± 0.3	0.2 ± 0.5
Dead fetuses (mean ± S.D)	16.7 ± 32.1	6.5 ± 10.8	7.3 ± 16.6	17.1 ± 31.9
Live fetuses (mean ± S.D)	6.5 ± 3.4	8.1 ± 2.1	7.7 ± 2.7	6.4 ± 3.1
Sex ratio (M : F)	54 : 46	47 : 53	51 : 49	51 : 49
Fetal weights (all viable fetuses)	43.02 ± 6.54	43.05 ± 4.72	42.06 ± 6.44	43.53 ± 6.03
Fetal weights (male fetuses)	43.35 ± 7.13	43.08 ± 4.55	43.20 ± 6.18	43.72 ± 8.07
Fetal weights (female fetuses)	41.50 ± 5.32	42.08 ± 4.90	40.84 ± 4.83	42.89 ± 5.11

Offspring:

There were no incidences of fetal external variations in any group. One fetus in the 0.01 mg/kg/day dose group had malrotated hind limbs and another fetus in the 0.1 mg/kg/day dose group had umbilical hernia. The following soft tissue and skeletal variations were observed with higher incidences in the treated groups: the incidences of small or missing intermediate lobe of the lung were higher in the treatment groups; incomplete sternebral ossification was observed in 3 fetuses (2 litters) of the low dose group; the incidences of 13^{th} rudimentary ribs and 13^{th} unilateral full ribs were higher in the treatment groups; 2 fetuses in the mid dose group had thickened ribs. However, none of these incidences were dose dependent, and similar to the incidences observed in this strain of rabbits from the conducting laboratory.

Fetal soft tissue and skeletal variations/malformations in different groups are summarized in the Table below.

Parameter	**0 mg/kg/day**	**0.01 mg/kg/day**	**0.03 mg/kg/day**	**0.10 mg/kg/day**
Litters evaluated	15	17	19	16
Fetuses evaluated	111	137	146	116
Fetal Soft Tissue Variations				
Variations of the major vessels				
- Fetal incidence	3 (2.7%)	4 (2.9%)	2 (1.4%)	3 (2.6%)
- Litter incidence	3 (20%)	4 (24%)	2 (11%)	3 (19%)
Intermediate lobe of the lung small/missing				
- Fetal incidence	1 (0.9 %)	7(5.1%)	2 (1.4%)	2 (1.7%)
- Litter incidence	1 (6.7%)	4 (24%)	2 (11%)	2 (13%)
Fetal Soft Tissue Malformations				
Heart and/or great vessel malformations				
- Fetal incidence	1 (0.9%)	1 (0.7%)	0 (0.0%)	0 (0.0%)
- Litter incidence	1 (6.7%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Fetal Skeletal Variations				
Incomplete ossification of hyoid body				
- Fetal incidence	0 (0.0%)	1 (0.7%)	0 (0.0%)	0 (0.0%)
- Litter incidence	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Accessory bone(s) in skull				
- Fetal incidence	1 (0.9%)	1 (0.7%)	0 (0.0%)	1 (0.9%)
- Litter incidence	1 (6.7%)	1 (5.9%)	0 (0.0%)	1 (6.2%)
26 presacral vertebrae				
- Fetal incidence	28 (25%)	37 (27%)	37 (25%)	19 (16%)
- Litter incidence	10 (67%)	9 (53%)	12 (63%)	9 (56%)
Misaligned or bipartite distal caudal vertebra (e)				
- Fetal incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)
- Litter incidence	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.2%)
5^{th} /6^{th} sternebra incomplete ossification				
- Fetal incidence				

- Litter incidence	29 (26%) 12 (80%)	33 (24%) 15 (88%)	37 (25%) 14 (74%)	26 (22) 14 (88%)
Other sternebra (e) incomplete ossification - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	3 (2.2%) 2 (12%)	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)
6th sternebra bifurcated - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	1 (0.7%) 1 (5.9%)	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)
13th full rib(s) - Fetal incidence - Litter incidence	66 (59%) 14 (93%)	59 (43%) 15 (88%)	71 (49%) 18 (95%)	59 (51%) 15 (94%)
13th rudimentary rib(s) - Fetal incidence - Litter incidence	11 (9.9%) 7 (47%)	22 (16%) 16 (94%)	24 (16%) 11 (58%)	17 (15%) 8 (50%)
13th unilateral full rib(s) - Fetal incidence - Litter incidence	2 (1.8%) 2 (13%)	6 (4.4%) 4 (24%)	9 (6.2%) 7 (37%)	5 (4.3%) 5 (31%)
Thickened rib(s) - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)	2 (1.4%) 2 (11%)	0 (0.0%) 0 (0.0%)
Fetal skeletal malformations				
Vertebral anomaly with/without associated rib anomaly - Fetal incidence - Litter incidence	1 (0.9%) 1 (6.7%)	3 (2.2%) 3 (18%)	0 (0.0%) 0 (0.0%)	1 (0.9%) 1 (6.2%)
Misaligned, fused and/or absent caudal vertebra (e) - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)	1 (0.9%) 1 (6.2%)
Fibula(e) absent - Fetal incidence - Litter incidence	0 (0.0%) 0 (0.0%)	1 (0.7%) 1 (5.9%)	0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%)

Summary:

In the segment II teratogenicity study with RU-0211 in rabbits, groups of pregnant females received 0, 0.01, 0.03 and 0.10 mg/kg/day doses of the drug from Gestation Day 7 through Gestation Day 20. RU-0211 was not teratogenic in rabbits at oral doses up to 0.10 mg/kg/day.

Study title: Segment III Pre- and Post-natal Development Study in Rats after Oral Administration of RU-0211 Minicapsules.

Key study findings: In the Segment III pre- and post-natal development study in rats, treatment of the F_0 female animals with RU-0211 had no significant effect on the pre- and post-natal development of F_1 animals. Gestation period was prolonged for the high dose F_0 dams, and had increased number of litters with stillborn pups. Half of the high dose pregnant dams had total litter death following parturition. The F_1 pups from the high dose group had decreased body weights and the female pups had decreased open field activity on the day following weaning (Day 22). Treatment with RU-0211 of F_0 females had no effects on mating and reproductive performance of the F_1 animals.

Study Report No: SPI/SR02-010; Cross reference #7142-105

Conducting laboratory and location:

Date of study initiation: February 27, 2001

GLP compliance: Yes

QA reports: **yes (X) no ( )**

Drug, lot #, radiolabel, and % purity: RU-0211, Lot Numbers 010221-2, 010221-3, 010221-4

Formulation/vehicle: RU-0211 was diluted with medium chain fatty acid triglyceride (MCT) and formulated into minicapsules.

Methods:

Species/strain: CD (SD)IGS BR rats.

Doses employed: Ru-0211 was formulated as minicapsules and administered at oral doses of 0, 0,02, 0.2 and 1.0 mg/kg/day. The doses were selected on the basis of a previously conducted developmental toxicity study in rats in which groups of pregnant animals received RU-0211 at 0, 0.016, 0.08, 0.4 and 2.0 mg/kg/day doses on gestation days 6

through 17. The high dose animals had loose stools and decreased body weight gains (62%). So, for the pre- and post-natal developmental toxicity study, the sponsor selected 1.0 mg/kg/day as the high dose.

Route of Administration: RU-0211 minicapsules were administered to the animals by the oral route.

Study Design: The drug was administered orally as minicapsules to pregnant animals from gestation day 6 through lactation day 20. The doses were administered once a day based on the most recent body weights. The F_0 females were observed daily for clinical signs and mortality. The body weights were recorded on gestation days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20 and on lactation days 0, 4, 7, 10, 14, 17 and 21. The dams were allowed to raise their F_1 pups to Day 21 postpartum. At the completion of weaning (lactation Day 21), one pup/sex/litter was randomly selected and maintained until the beginning of the maturation phase (Days 30 to 35 postpartum). All other pups were sacrificed and discarded. After 7-week post-weaning phase, male and female F_1 animals were cohabited for up to 21 days for mating. After the mating period, the F_1 females were allowed to deliver and as soon as possible after the birth, the live and dead F_2 pups were sexed and the live pups were examined for gross abnormalities. The dead F_2 pups were examined for gross abnormalities of cervical, thoracic and abdominal viscera and preserved in neutral-buffered formalin. The F_1 males and females were sacrificed and examined for gross abnormalities.

Number/sex/group: 25 mated females/group

Parameters and endpoints evaluated:

F_0 females: The F_0 females were observed twice daily for clinical signs and mortality. The body weights and food consumption were recorded at regular intervals. Necropsies were performed on all dead and sacrificed F_0 females. The uteri and ovaries were examined for implantations and corpora lutea, respectively. The animals that failed to give birth to viable litters were sacrificed and examined for gross abnormalities. The presence of early or late resorptions, live or dead fetuses and the implantation sites were recorded.

F1 animals: After delivery, the F_1 pups were sexed and examined for external abnormalities. On lactation days 0, 4, 7, 14 and 21, the number of live pups per sex per litter, body weights and clinical observations were recorded. The following developmental landmarks were evaluated for each pup: pinna unfolding (beginning on Day 1), surface righting reflex (beginning on Day 4, hair growth (beginning on Day 7), incisor eruption (beginning on Day 7), eye opening (beginning on Day 11) and auditory startle (Day 21). At the end of weaning, one F_1 pup/sex/litter was randomly selected and maintained until the maturation phase was reached. The F_1 animals were examined for vaginal opening or cleavage of the balanopreputial gland beginning on Days 30 and 35 postpartum, respectively. Locomotor activity, pupillary reflex and water maze tests for learning and memory assessments were conducted on all selected animals. Following the 7-week post-weaning maturation phase, F_1 males and females were paired for mating for up to 21 days. The F_1 females were allowed to deliver the pups. The dead animals were sacrificed and examined for gross abnormalities. The non-pregnant females and the females that delivered were sacrificed and necropsies performed. The F_1 males were also sacrificed and examined macroscopically. The abnormalities from all animals were preserved in buffered formalin.

F_2 pups: Soon after the birth, each dead and live pup was sexed, weighed and examined for external abnormalities. Dead pups were examined for cervical, thoracic and visceral abnormalities and preserved in alcohol. On lactation Day 2, the F_2 pups were killed and preserved in buffered formalin.

Results:

Mortality: Two F_0 females, each in the control (#B37978 and #B38002) and 1.0 mg/kg/day group (#B38019 and #B38021), were found dead during the gestation period; the deaths were not related to treatment. One female each in 0.02 (#B38029) and 0.2 (B38060) mg/kg/day groups died and the deaths were considered accidental. In addition, 3 females in the 1.0 mg/kg/day group (B38041, B38042 and B38072) died during parturition.
One female each in the 0.2 (B38010) and 1.0 (B38020) mg/kg/day died during lactation. The cause of the deaths is not known. One control female (B38023) also died during the lactation period.

Clinical signs: Treatment-related clinical signs were observed only in the high dose F_0 females and included salivation, urine stains and soft feces. One female each in 0.02 and 1.0 mg/kg/day group was not pregnant and one female in the 1.0 mg/kg/day group had early resorption of the entire litter. There was total litter death in one 0.2 mg/kg/day and 12 high dose dams. Clinical signs of weakness, paleness, cold to touch, thin and no visible milk in the stomach were observed in these litters before death.

Body weight: The mean body weights of the control F_0 females on gestation Days 0 and 20 were 227.2±11.5 and 374.5±24.6 g, respectively. The body weight gains for the high dose females were 28.4% and 34.6% lower than that of controls during gestation days 0 to 20 and 6 to 20, respectively. The body weight gains of the low, mid and high dose females from Days 6 to 20 of gestation were 100%, 97.8% and 65.4% of control, respectively. The body weight gains for the mid and high dose groups were higher than that of controls during lactation Days 0-20. The body weight gains for low, mid and high dose females from Days 0 to 20 of lactation were 81.8%, 124.4%, and 243.3% of the control, respectively.

Food consumption: The mean food consumption of the control F_0 females on gestation days 4-6 and 16-18 were 26.8±11.4 and 30.5±2.8 mg/kg/day, respectively. Food consumption of females receiving the 1.0 mg/kg/day dose was lower than that of controls during gestation days 6-12 and 16-18. There were 54.0%, 21.3% and 11.5% decreases in food consumption on gestation days 6-8, 10-12 and 16-18, respectively. During lactation days 0-20, the food consumption of the mid dose F_0 females were higher, which corresponds to slightly increased body weights during this period.

In-life observations:

Dams: Three F_0 dams from 1.0 mg/kg/day group died during delivery on gestation day 23. The duration of gestation was longer for this group, and there was complete resorption of one litter. The high dose animals also had increased number of litters with stillborn pups and with

complete litter loss prior to week-4. The delivery and litter data for F_0 dams are summarized in the Table below.

Parameters	0 mg/kg/day	0.02 mg/kg/day	0.2 mg/kg/day	1.0 mg/kg/day
Number pregnant (% pregnancy)	25 (100%)	24 (96%)	25 (100%)	24 (96%)
Number of F_0 dams that delivered	23 (92%)	23 (92%)	24 (96%)	18 (72%)
Gestation period, days	21.8	21.7	21.7	22.1
Dams with stillborn pups	0 (0%)	2 (8.7%)	2 (8.3%)	5 (28%)
Number of pups delivered	13.57± 2.02	12.83± 2.37	13.5± 2.11	11.89±3.5
Number of stillborn	0	3	2	8
Number of live born	312	292	322	206
Number cannibalized	0	0	0	2
Live birth index	100	99	99	95
Viability index (mean %)	94	99	94	25
Implantation sites	14.09	14.09	14.04	14.78
Pups surviving at Day 21	175	180	175	33
Sex distribution, % [M/(M + F)]	54	52	49	55
Pup weight/litter (G)	6.31±0.50	6.62±0.50	6.30±0.72	5.19±0.97

Offspring:

No external malformations were evident for F_1 fetuses. No treatment-related clinical signs were observed in any groups. The mean body weights of the F_1 male and female pups from the high dose group were lower during the weaning period (10.5% and 9.9%, respectively on Day 1). However, after Day 7, the body weights of the high dose group pups were only slightly decreased (3.3 - 5.6% in males and 2% - 3.4% in females).

No treatment-related effects on physical (preputial separation, vaginal opening, eye opening, hair growth or incisor eruption) or functional (pinna folding, auditory reflex, pupillary reflex or surface righting reflex) development of F_1 pups were observed in any group. No treatment-related clinical signs were observed in F_1 males and females during the gestation or lactation period.

Behavioral development of F_1 rats was assessed with the open field test (Day 22 postpartum and Week 5 post-weaning) and the water "M" maze test. There were no differences in the open field testing mean activity for F_1 male control and treatment groups. For F_1 females of 0.2 (at 16-20 minutes, 96.65% reduction) and 1.0 (at 11-15 and 16-20 minutes, 60.7% and 96.9% reductions, respectively) mg/kg/day groups, the mean activity was significantly reduced on Day 22 postpartum. For the water maze learning and reversal learning tests, there were no differences between the control and treatment groups.

No effects on mating and fertility indexes were evident for F_1 male and female rats. Gestation period was slightly prolonged in the 0.02 and 0.2 mg/kg/day doses, however, no effect was observed at 1.0 mg/kg/day. There were no treatment-related effects on body weight gains for F_1 mated female rats from days 0 to 21 of gestation. The pregnancy and litter data for F_1 females is summarized in the Table below.

Parameters	0 mg/kg/day	0.02 mg/kg/day	0.2 mg/kg/day	1.0 mg/kg/day
Number mated	20	23	21	16
Number pregnant (% pregnancy)	20 (100%)	22 (96%)	19 (90%)	15 (94%)
Number of F_0 dams that delivered	20 (100%)	22 (96%)	19 (90%)	15 (94%)
Gestation period, days	21.6±0.6	22.0±0	22.1±0.3	21.6±0.5
Dams with stillborn pups	2 (10%)	1 (4.5%)	2 (11%)	3 (20%)
Number of pups delivered	15.05± 2.87	14.09± 2.94	14.21± 3.82	14.33± 2.64
Number of stillborn	2	1	2	3
Number of live born	299	309	268	212
Implantation sites	15.60±3.03	15.00± 2.65	15.21± 3.97	15.60± 1.35
Pup weight/litter (G)				
- males	6.39±0.45	6.63±0.66	6.45±0.49	6.41±0.73
- females	6.06±0.50	6.24±0.68	6.06±0.46	6.01±0.61
External malformations	0	0	0	0

Terminal and Necroscopic Evaluations:

Dams: Gross pathological examination of F_0 dams following the end of lactation revealed raised areas in the stomachs of the mid (2/25) and high (17/25) dose animals. The incidence of gravid uterus was higher in the high dose females (control 2/25, low dose 1/25, mid dose 2/25 and high dose 6/25).

Offspring: Visceral examination of the F_1 pups killed on lactation day 4 or those died during this period did not show any treatment-related abnormalities. Gross pathology examinations of F_1 males, used for mating, showed higher incidences of dilated kidney pelvis (control 1/22, low dose 3/23, mid dose 6/22, high dose 4/16) and distended urinary bladders (control 0/22, low dose 1/23, mid dose 1/22, high dose 1/16) in the treatment groups. F_1 females, used for reproduction, had no treatment-related gross abnormalities. There was no evidence of treatment-related external malformations in F_2 fetuses.

Summary: In the Segment III pre- and post-natal development study in rats, pregnant female animals received 0, 0.02, 0.2 and 1.0 mg/kg/day doses of RU-0211 from gestation day 6 through lactation day 20. Treatment with RU-0211 had no significant effect on the pre- and post-natal development of F_1 rats. The high dose F_0 females had decreased body weight gains during the gestation period and half of the pregnant dams had total litter death following parturition. Gestation period was prolonged for the high dose F_0 dams, and had increased number of litters with stillborn pups. The F_1 pups from the high dose group had decreased body weights and the female pups had decreased open field activity on the day following weaning (Day 22). There were no treatment-related findings in the F_1 offspring following maturation, and RU-0211 treatment had no effects on mating and reproductive performance of the F_1 animals.

2.6.6.7 Local tolerance

Study title: Antigenicity Study of RU-0211 with Guinea Pigs (Final Study Report).

Study Report No: RTU/SR00-011 (Cross reference number: Study No. 800919)
Site and testing facility:

Date Started: August 10, 1999

Date of Study Report: December 10, 1999 (May 19, 2000) (Translation from Japanese to English January 4, 2000)

GLP compliance: This study was conducted in compliance with GLP standards specified in the Japanese Ministry of Health and Welfare Ordinance No. 21 (March 26, 1997).

QA- Report Yes ( ) No (X): There were no statements regarding compliance with the Quality Assurance Unit.
Lot and batch numbers: RU-0211, Lot No. 9

Methods: The antigenicity of RU-0211 was evaluated by the active systemic anaphylaxis (ASA) assay in guinea pigs and the passive cutaneous anaphylaxis (PCA) assay in guinea pigs with sera obtained from sensitized guinea pigs.

- **Species/strain:** Male Hartley Guinea pigs (SPF) were obtained from Animals were approximately 5 weeks old at the start of treatment.
- **Route of Administration:** For the ASA assay, guinea pigs received RU-0211 by the oral or subcutaneous route. The positive control group received egg albumin + Freund's complete adjuvant (FCA) by the subcutaneous route
- **Doses employed for ASA Assay:** RU-0211 was administered by the oral route at doses of 2 or 20 μg/kg. For a separate group of animals, RU-0211 at a dose of 20 μg/kg in combination with FCA was administered by the subcutaneous route. Egg albumin was administered at a subcutaneous dose of 2 mg/kg. Sensitization by the oral route of administration was performed 5 times per week for 3 consecutive weeks (i.e., total of 15 treatments). Sensitization by the subcutaneous route was performed once per week for 3 consecutive weeks (i.e., total of 3 treatments). The challenge antigen (i.e., 10 μg RU-0211 for RU-0211-sensitized animals, 2 mg/kg egg albumin to egg albumin-sensitized animals, and RU-0211 or egg albumin to untreated controls) was administered to animals by the intravenous route at 14 days after the final sensitization treatment.
- **Rationale for ASA Assay:** In preliminary tests, it was determined that RU-0211 at an oral dose of 20 μg/kg or an intravenous dose of 10 μg/animal produced no abnormal signs in male guinea pigs.

-PCA Assay: Blood was collected from sensitized animals on the day prior to challenge and serum was prepared. Untreated guinea pigs received intradermal injection of sensitized serum at 0.5 mL/site on the back. Serum obtained from RU-0211-treated animals was not diluted. Serum from egg albumin + FCA-treated animals was diluted 30, 100, 300, 1000, or 3000 times. The

challenge antigen (i.e., 10 μg RU-0211 for RU-0211-sensitized animals, 2 mg/kg egg albumin to egg albumin-sensitized animals, and RU-0211 or egg albumin to untreated controls) plus Evan's blue dye was administered by the intravenous route at 4 hr after serum sensitization.

- **Number of animals/sex/dosing group:** For the ASA assay, there were 5 male guinea pigs/group in each RU-0211 treatment group, 3 male guinea pigs in the positive control group, and 6 male guinea pigs in the untreated control group. For the PCA assay, there were two guinea pigs per sensitized serum.
- **Endpoints:** For the ASA assay, animals were observed for signs of anaphylaxis within 30 min after the challenge dose and mortality within 24 hr after the challenge dose. For the PCA assay, animals were sacrificed 30 min after administration of challenge antigen and the dorsal skin was removed. The size of the blue spot induced by leakage of Evan's blue dye was measured and spots >5 mm on average taken in 2 animals were considered PCA positive. PCA titers were indicated by the maximum dilution factor of a serum which produced positive reactions.

Results:

- **Observations:** In the ASA assay, intravenous challenge with RU-0211 produced no anaphylactic symptoms in animals that received sensitization with RU-0211 by the oral or subcutaneous routes. For guinea pigs sensitized with RU-0211 at an oral dose of 20 μg/kg or a subcutaneous dose of 20 μg/kg (+FCA), evacuation of feces or micturition were observed in 2 of 5 and 1 of 5 animals, respectively, after challenge. Intravenous challenge with the positive control, egg albumin, in egg albumin-sensitized animals produced anaphylactic symptoms (i.e., fur erection, weak or diminished muscle tone, labored breathing, sneezing or coughing, retching, rales, evacuation of feces or micturition, convulsions, and prostration) and death in 2 of 3 animals. Challenge with RU-0211 or egg albumin in untreated animals produced evacuation of feces or micturition in 2 of 3 and 1 of 3 animals, respectively. For the PCA assay, challenge with RU-0211 caused no leakage of dye at any sites treated with serum from animals sensitized with RU-0211 by the oral or subcutaneous routes. Challenge with the positive control, egg albumin, produced leakage of dye at sites treated with serum from egg albumin-sensitized animals (titer ≥ 3000). Challenge with RU-0211 or egg albumin produced no leakage of dye at sites treated with serum from untreated animals.

Summary: RU-0211 was found to possess no antigenic potential as assessed by active systemic anaphylaxis (ASA) in guinea pigs and passive cutaneous anaphylaxis (PCA) in guinea pigs with sera obtained from sensitized guinea pigs.

2.6.6.8 Special toxicology studies

Study Title: Dose Range Toxicity Study of RU-0211 in Pregnant Guinea Pigs **(Study Protocol #4619-001)**

Methods: The purpose of this study was to find information for the selection of dosage for the oral abortion/resorption study of RU-0211 in pregnant guinea pigs. Sixty time-mated female Dunkin Hartley guinea pigs were randomly assigned to five dosage groups (Groups I through V; 12 animals per group), and each group was administered 0, 5, 20, 40 and 80 µg/kg/day doses (as capsules) of RU-0211on gestation days 40 through 53. The animals were observed twice daily for clinical signs, abortions, premature deliveries and mortality. The body weights were recorded pre-dose and daily during the dosing and post-dosing periods. Blood samples were collected from each surviving animal prior to initiation of dosing (gestation day 39), prior to the last dose administration (gestation day 53) and prior to euthanasia for determination of serum progesterone levels. All surviving guinea pigs were sacrificed on gestation day 60, cesarean-sectioned and gross necropsy performed. The number and distribution of corpora lutea were recorded, and the uterus of each animal was examined for pregnancy, number and distribution of implantation sites, live and dead fetuses, and early and late resorptions

Results: There were 11, 9, 10, 11 and 11 pregnant guinea pigs in the control, 5, 20, 40 and 80 µg/kg/day dosage groups, respectively. Abortions were observed in 4 (of 11) and 6 (of 11) guinea pigs in the 40 and 80 µg/kg/day dosage groups, respectively and were sacrificed. In the 40 mg/kg group, abortions occurred in two animals after 5 doses, and 2 animals aborted after 13 doses. In the 80 mg/kg group, abortions occurred after 4 (2 animals), 5 (1 animal), 6 (1 animal), 7 (1 animal) and 8 (1 animal) doses. In the 5 µg/kg/day group, one guinea pig was sacrificed moribund, and two were sacrificed due to abortion (one after 2 doses, and the other after 9 doses). In addition, 1, 2 and 3 animals from 5, 40 and 80 µg/kg/day groups had late resorptions of the fetuses. Mortality occurred in 3 (2 found dead, 1 sacrificed moribund) and 4 (3 found dead, 1 sacrificed moribund) guinea pigs receiving 40 and 80 µg/kg/day doses, respectively. No mortalities or abortions were observed in the 20 µg/kg/day dosage group. The mortalities of animals in different groups are shown in the Table below.

Mortality:

Dosage Group	0 µg/kg/day	5 µg/kg/day	20 µg/kg/day	40 µg/kg/day	80 µg/kg/day
Found Dead	0	0	0	2	3
Sacrificed Moribund	0	1	0	1	1
Total	**0**	**1**	**0**	**3**	**4**

The number of abortions and late resorptions in different groups is shown in the Table below.

Dosage Group	0 µg/kg/day	5 µg/kg/day	20 µg/kg/day	40 µg/kg/day	80 µg/kg/day
Abortions	0/11	2/9	0/10	4/11	6/11
Late Resorptions	0/11	1/9	0/10	2/11	3/11

Animals receiving 40 and 80 µg/kg/day doses showed the following clinical signs: decreased motor activity, abnormal fecal output, lacrimation, urine-stained abdominal fur, apparent dehydration, coldness to touch, respiratory difficulties, impaired or lost righting reflex, clonic convulsions, twitches, comatose, excess salivation, tremors and/or enophthalmos. In addition, red substance in the nesting box and/or red perivaginal substance were observed in some guinea pigs. Some of these signs were also observed in animals receiving the 5 µg/kg/day dose, and were generally associated with abortion or death.

The body weights of animals receiving 5, 20, 40 and 80 µg/kg/day doses were lower than controls. However, the mean body weights of the treatment group animals were also lower that that of controls before initiation of dosing (Days 39 and 40). The mean body weights of animals from different groups are shown in the Table below.

DOSE GROUP DOSE (MCG/KG/DAY)a		I 0 (VEHICLE)	II 5	III 20	IV 40	V 80
GUINEA PIGS TESTED	N	12	12	12	12	12
PREGNANT	N	11	9	10	11	11
MATERNAL BODY WEIGHT (G)						
DAY 0	MEAN±S.D.	932.6 ± 71.6	917.1 ± 96.5	933.7 ± 68.8	927.1 ± 85.3	943.1 ± 72.7
DAY 39	MEAN±S.D.	1010.8 ±101.7	937.3 ± 89.6	919.4 ± 89.6	951.4 ± 69.0	954.7 ± 74.6
DAY 40	MEAN±S.D.	1013.2 ±108.4	933.0 ± 98.3	922.9 ± 88.3	956.9 ± 79.8	957.1 ± 74.1
DAY 41	MEAN±S.D.	1014.4 ±131.7	921.3 ±102.4	899.5 ± 92.9	934.4 ± 70.8	909.3 ± 65.0
DAY 42	MEAN±S.D.	1044.7 ±122.9	881.6 ± 86.1	900.4 ± 98.6	882.6 ± 78.3	858.0 ± 52.7
DAY 43	MEAN±S.D.	1057.2 ±125.5 [8]b	872.4 ± 94.8	910.9 ± 95.9	880.1 ± 80.8	836.3 ± 47.8
DAY 44	MEAN±S.D.	1062.5 ±126.3	867.5 ±109.1 [8]b	916.5 ± 97.8	870.4 ± 93.6	816.2 ± 43.0 [9]b
DAY 45	MEAN±S.D.	1072.0 ±133.0	868.2 ±121.6 [8]b	922.3 ± 94.7	861.7 ± 96.7	787.0 ± 75.4 [5]b
DAY 46	MEAN±S.D.	1092.1 ±138.6	882.0 ±126.9 [8]b	926.9 ± 97.2	886.0 ±111.4 [9]b	751.0 ±124.0 [4]b
DAY 47	MEAN±S.D.	1108.7 ±142.4	885.9 ±143.7 [8]b	940.8 ±102.6	920.0 ±100.0 [7]b	776.0 ±114.7 [3]b
DAY 48	MEAN±S.D.	1122.0 ±151.2	922.1 ±144.5 [7]b	955.7 ±102.6	915.3 ± 97.5 [7]b	817.5 ±118.1 [2]b
DAY 49	MEAN±S.D.	1134.9 ±149.8	930.0 ±131.2 [7]b	959.7 ±109.0	909.7 ± 88.4 [7]b	919.0 ± 0.0 [1]b
DAY 50	MEAN±S.D.	1145.3 ±152.3	953.0 ± 82.2 [6]b	966.2 ±117.0	909.0 ± 93.7 [7]b	926.0 ± 0.0 [1]b
DAY 51	MEAN±S.D.	1157.8 ±155.4	962.2 ±102.3 [6]b	974.8 ±112.5	904.0 ± 95.4 [7]b	932.0 ± 0.0 [1]b
DAY 52	MEAN±S.D.	1171.1 ±161.1	976.7 ±110.9 [6]b	978.6 ±107.6	916.0 ± 97.9 [6]b	943.0 ± 0.0 [1]b
DAY 53	MEAN±S.D.	1187.6 ±161.4	1007.0 ±101.4 [6]b	984.9 ±101.8	906.2 ± 52.2 [5]b	939.0 ± 0.0 [1]b

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 40 through 53 of gestation.
b. Excludes values for sows that were found dead, moribund sacrificed or aborted.

The mean progesterone levels of the animals were higher in all groups, and treatment with RU-0211 was not associated with any changes in the mean progesterone levels in any group. The progesterone levels of individual animals are shown in the Table below.

TABLE 14 (PAGE 1): PROGESTERONE LEVELS (NG/ML) - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 39	53	60
GUINEA #	DOSE GROUP I		0 (VEHICLE) MCG/KG/DAY
780 P	119.40	157.40	151.30
4327 P	270.90	208.20	183.90
4583 P	255.20	228.70	176.80
2318 P	437.10	245.50	258.40
19 P	279.90	323.90	202.30
1326 P	383.30	181.70	99.10
2302 P	159.60	168.20	146.30
8869 P	248.20	259.80	161.50
779 NP	0.32	0.00	2.52
1273 P	159.10	286.30	295.50
596 P	250.00	405.20	218.70
2790 P	397.60	141.00	126.40
GUINEA #	DOSE GROUP II		5 MCG/KG/DAY
7515 P	467.90	230.50	207.80
5043 P	106.80	241.20	147.40
4351 NP	2.24	0.16	2.88
3770 NP	0.40	2.68	0.20
7280 NP	4.00	0.00	1.52
6005 P	351.90	157.00	173.10
6053 P	195.30	135.70	258.50
9885 P	256.40	MORIBUND SACRIFICED ON DAY 47 OF GESTATION	
6513 P	205.90	170.60	169.10
1245 P	388.90	204.20	276.90
1869 P	425.40	ABORTED AND SACRIFICED ON DAY 42 OF GESTATION	
3613 P	308.90	ABORTED AND SACRIFICED ON DAY 49 OF GESTATION	

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION

TABLE 14 (PAGE 2): PROGESTERONE LEVELS (NG/ML) - INDIVIDUAL DATA

PREGNANCY STATUS	DAY 39	53	60
GUINEA #	DOSE GROUP III		20 MCG/KG/DAY
9617 P	478.50	227.00	208.40
5054 P	90.30	177.90	199.90
6856 P	372.40	174.40	159.70
8515 NP	71.40	6.68	0.48
2848 P	296.80	165.60	135.90
1532 P	47.60	354.70	272.30
3597 P	138.30	291.40	248.10
5813 P	286.50	208.30	175.60
9343 NP	1.48	0.12	1.00
4624 P	68.90	168.20	282.40
1626 P	111.50	200.60	160.60
7133 P	64.00	301.00	265.50
GUINEA #	DOSE GROUP IV		40 MCG/KG/DAY
7359 P	305.40	FOUND DEAD ON DAY 53 OF GESTATION	
3264 P	138.30	ABORTED AND SACRIFICED ON DAY 45 OF GESTATION	
1846 P	465.40	FOUND DEAD ON DAY 46 OF GESTATION	
1865 P	317.20	MORIBUND SACRIFICED ON DAY 46 OF GESTATION	
2005 P	362.70	ABORTED AND SACRIFICED ON DAY 51 OF GESTATION	
4315 P	6.10	44.20	48.90
558 P	126.50	435.50	302.60
1260 P	216.50	436.20	326.60
7811 P	437.20	ABORTED AND SACRIFICED ON DAY 53 OF GESTATION	
2792 NP	2.92	0.40	3.00
9673 P	331.10	ABORTED AND SACRIFICED ON DAY 45 OF GESTATION	
6657 P	257.10	370.10	369.20

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION

TABLE 14 (PAGE 3): PROGESTERONE LEVELS (NG/ML) - INDIVIDUAL DATA

PREGNANCY STATUS	DAY	39	53	60
GUINEA #		DOSE GROUP V		80 MCG/KG/DAY
8112	P	131.60	132.10	94.90
6328	P	526.80	FOUND DEAD ON DAY 45 OF GESTATION	
5105	P	43.50	ABORTED AND SACRIFICED ON DAY 45 OF GESTATION	
6377	P	240.90	MORIBUND SACRIFICED ON DAY 44 OF GESTATION	
1842	P	273.40	FOUND DEAD ON DAY 44 OF GESTATION	
4082	P	99.10	ABORTED AND SACRIFICED ON DAY 47 OF GESTATION	
1608	P	194.70	FOUND DEAD ON DAY 44 OF GESTATION	
3633	P	299.00	ABORTED AND SACRIFICED ON DAY 44 OF GESTATION	
541	NP	1.92	0.00	0.88
9999	P	278.20	ABORTED AND SACRIFICED ON DAY 44 OF GESTATION	
3243	P	83.50	ABORTED AND SACRIFICED ON DAY 48 OF GESTATION	
21	P	597.30	ABORTED AND SACRIFICED ON DAY 46 OF GESTATION	

P = PREGNANT NP = NOT PREGNANT (VALUES EXCLUDED FROM AVERAGES)
DAY = DAY OF PRESUMED GESTATION

Thus, an increase in abortion and post-implantation loss occurred at RU-0211 doses of 40 and 80 µg/kg/day doses. The mean litter size, live litter size and the percentage of guinea pigs with viable fetuses were lower than controls in the 40 and 80 µg/kg/day groups.

From these findings, 1, 10 and 25 µg/kg/day doses were selected for the definitive abortion/resorption study in guinea pigs.

Study title: Oral (Capsule) Abortion Study of RU-0211 in Guinea Pigs

Key study findings: The abortifacient potential of RU-0211 was examined in guinea pigs after oral administration of the drug at oral doses of 1, 10 and 25 µg/kg, on gestation days 40 through 53. Abnormal clinical signs were observed in animals receiving the 25 µg/kg dose. Treatment-related mortalities were observed at the 25 µg/kg dose. Two (of 24) animals from the 10 µg /kg, and 5 (of 24) animals from the 25µg /kg main study group had abortions. In addition, 2 animals (of 5) each from the 1 µg /kg and 10 µg /kg satellite groups had abortions. There were no abortions in any of the control groups. Thus, RU-0211 was abortifacient in guinea pigs.

Study Report no.: SPI/SR05-009 (Cross reference # 4619-002)
Conducting laboratory and location: —

Date of study initiation: June 15, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: RU-0211; Lot Numbers: YY040621-2 (2 µg/ml), YY040621-3 (20 µg/ml), YY040621-4 (50 µg/ml).
Formulation/vehicle: RU-0211 was dissolved in medium chain fatty acid triglyceride (MCT; — lot # YY040621-1), and encapsulated in empty gelatin capsules (hard shell). Control animals were administered MCT.

Methods:

Doses: RU-0211 was administered orally at doses of 1, 10 and 25 µg/kg (0.5 ml/kg). The doses were selected on the basis of a dose range finding study in pregnant guinea pigs in which groups of animals received 5, 20, 40 and 80 µg/kg/day doses of the drug. There were mortalities and abortions at 40 and 80 µg/kg/day. From these findings, 1, 10 and 25 µg/kg/day doses were selected for the abortion/resorption study in guinea pigs.

Study design: The study was conducted in timed mated (HA)BR (Hartley) guinea pigs. The animals were about 5 to 18 months of age, and had body weights of 746 to 1042 g at study assignment. Five groups of mated female animals (two replicates of 12 animals/replicate; a total of 24 animals/group) were used in the study. There were two control groups; one untreated control, and the other control group received the vehicle. RU-0211 was administered at oral doses of 1, 10 and 25 µg/kg/day doses on gestation days 40 through 53. In addition, satellite groups consisting of 5 animals/group were used for serum progesterone measurements.
The animals were observed twice daily for clinical signs and mortality during the dosing period. The animals were also examined for abortions, premature deliveries and deaths before dose administration and 60 ± 10 minutes after dose administration. Body weights were recorded on gestation days 0, 15, 18, 21, 24, 27, 30, 33, 36 and 39, and daily during the dosing and post-dosing periods. Blood samples (1.5 ml) were collected from the satellite group animals on gestation days 39, 53 and 60 for determination of serum progesterone levels. Blood samples were also collected from animals found to be in moribund condition or aborting.
Pregnancy was terminated on gestation day 60 by cesarean section, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Pregnancy status and uterine contents of the animals were recorded. Aborted fetuses and conceptuses *in utero* of guinea pigs assigned to the main study groups were examined using the same method used for term fetuses. The uteri of apparently non-pregnant animals were examined to confirm the absence of implantation sites. The number and distribution of corpora lutea were recorded. The uterus of each guinea pig was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions.

Results:

Mortalities: There were mortalities (found dead or sacrificed moribund) in all groups, except the untreated control group. In the main study group, 1, 2, 0 and 3 animals were found dead in the vehicle control, 1, 10 and 25 µg/kg groups, respectively, and 1 animal from the 25 µg/kg group was sacrificed moribund. Deaths in the vehicle control and the 1.0 µg/kg groups occurred after 2-4 doses. Deaths and/or moribund sacrifices in the 25 µg/kg group occurred after 5 to 11 doses. One animal in the 1.0 mg/kg satellite group was found dead. Thus, the mortalities observed were not dose-related. Mortalities of animals in different groups are summarized in the Table below.

Mortalities:

	Assigned Group	Untreated	Vehicle	1.0 µg/kg/day	10 µg/kg/day	25 µg/kg/day
Found Dead	**Main**	**0**	**1**	**2**	**0**	**3**
	Satellite	**0**	**0**	**1**	**0**	**0**
Sacrificed Moribund	**Main**	**0**	**0**	**0**	**0**	**1**
	Satellite	**0**	**0**	**0**	**0**	**0**
Total	**Main plus Satellite**	**0**	**1**	**3**	**0**	**4**

Clinical signs: The 25 µg/kg dose of RU-0211 was associated with adverse clinical signs which included ungroomed coat, localized alopecia, cold to touch, red perivaginal substance and decreased motor activity. The observation of red substance in the nesting box or cage pan and/or red perivaginal substance was observed in 3 of 5 animals that aborted. Additional clinical signs observed in animals in the 25 µg/kg group that died, sacrificed or aborted included tremors, lacrimation, apparent dehydration, scant feces, abrasion of the mouth, excess salivation, edema and gasping (gasping was observed in one animal immediately following dosing). The 10 µg/kg dose was not associated with any abnormal clinical signs. Clinical signs observed in different groups of maternal animals are summarized in the Table below.

Clinical Signs

Observation	Control (Untreated)	Control (Vehicle)	1 µg/kg	10 µg/kg	25 µg/kg
Ungroomed coat	2/24	0/24	2/24	0/24	6/24
Localized alopecia	0/24	0/24	2/24	0/24	4/24
Cold to touch	0/24	0/24	0/24	0/24	3/24
Red perivaginal substance	0/24	0/24	0/24	0/24	3/24
Decreased motor activity	0/24	0/24	0/24	0/24	3/24
Tremors	0/24	0/24	0/24	0/24	2/24
Red perioral substance	0/24	0/24	0/24	0/24	2/24
Lacrimation	0/24	0/24	0/24	0/24	1/24
Dehydration	0/24	0/24	0/24	0/24	1/24
Scant feces	0/24	0/24	0/24	0/24	1/24

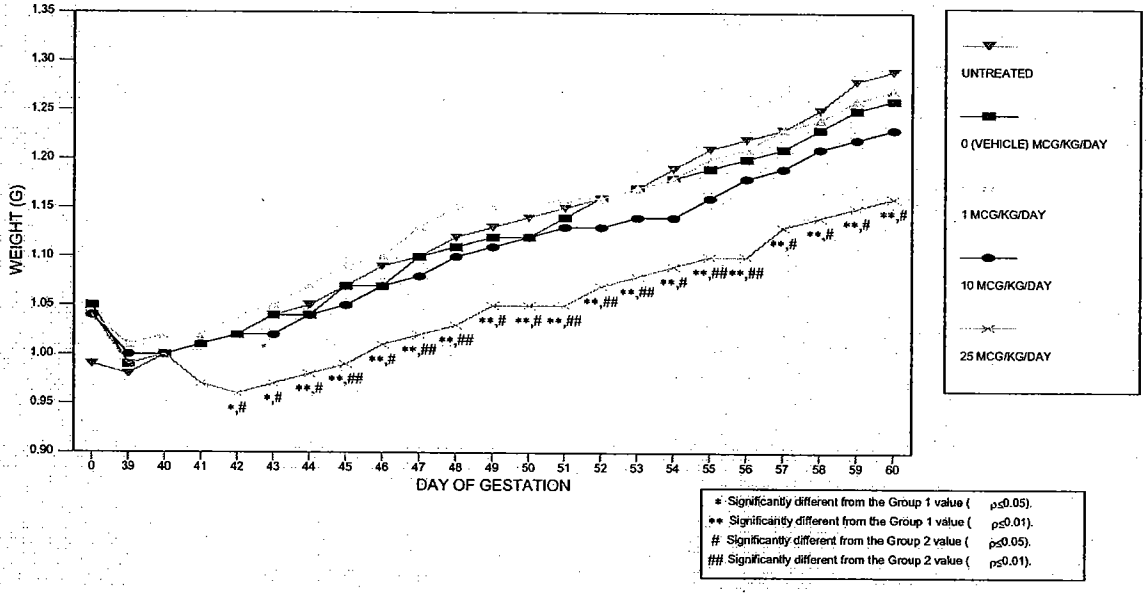
Body Weights and Food Consumption: The mean body weights of the untreated and vehicle control animals on gestation day 0 were 0.99±0.07 and 1.05±0.11 kg, respectively. The mean body weights of animals treated with the 25 µg/kg dose were lower than that of controls (<10%) during the dosing and gestation periods. The mean food consumption of animals receiving the high dose was lower than that of the vehicle control group during the dosing period. The mean food consumptions of the high dose group were 33%, 37%, 16%, 15% and 22% lower than the vehicle control group on gestation days 42, 45, 48, 51 and 54, respectively. The body weights of the maternal animals from different groups are shown in the Table and Figure below.

Body Weights

Time of gestation	Control (Untreated) 1	Control (Vehicle) 2	1 µg/kg 3	10 µg/kg 4	25 µg/kg 5
Day 0	0.99 ± 0.07	1.05 ± 0.11	1.04 ± 0.08 (99%)	1.04 ± 0.08 (99%)	1.04 ± 0.09 (99%)
Day 40	1.0 ± 0.07	1.0 ± 0.07	1.02 ± 0.06 (102%)	1.0 ± 0.07 (100%)	1.0 ± 0.08 (100%
Day 44	1.05 ± 0.08	1.04 ± 0.05	1.07 ± 0.08 (103%)	1.04 ± 0.08 (100%)	0.98 ± 0.10** ## (94%)
Day 48	1.12 ± 0.09	1.11 ± 0.06	1.15 ± 0.10 (103%)	1.10 ± 0.09 (98%)	1.03 ± 0.09** ## (93%)
Day 52	1.16 ± 0.09	1.16 ± 0.06	1.16 ± 0.10 (100%)	1.13 ± 0.10 (97%)	1.07 ± 0.06** ## (92%)
Day 54	1.19 ± 0.09	1.18 ± 0.06	1.18 ± 0.10 (100%)	1.15 ± 0.10 (98%)	1.09 ± 0.06** # (92%)
Day 58	1.25 ± 0.10	1.23 ± 0.06	1.24 ± 0.12 (101%)	1.21 ± 0.14 (97%)	1.14 ± 0.08** # (93%)
Day 60	1.29 ± 0.11	1.26 ± 0.07	1.27 ± 0.13 (101%)	1.23 ± 0.14 (98%)	1.16 ± 0.08** # (92%)

**, p=0.01 (compared with group 1); ##, p=0.01 (compared with group 2); #, p=0.05 (compared with control 2). Dosing occurred on days 40 through 53. The values inside the parenthesis are the percent of control (vehicle) body weights.

MATERNAL BODY WEIGHTS
REPLICATES A AND B COMBINED
Figure 1



Abortion: In the main study group, 2 (of 21) animals receiving the 10 µg/kg dose and 5 (of 23) animals receiving the 25 µg/kg dose had abortions. From the satellite groups, 1 (of 5) animal from the 1µg/kg group was found dead and 2 (of 5) animals each from the 1 and 10 µg/kg groups had abortions. The 2 abortions in the 10 µg/kg group occurred after 14 doses. These animals were gaining weight and had no abnormal clinical signs. In the 25 µg/kg group, abortions occurred after 8 to 14 doses. The number of abortions for guinea pigs in the main and satellite groups are summarized in the Table below.

Abortions

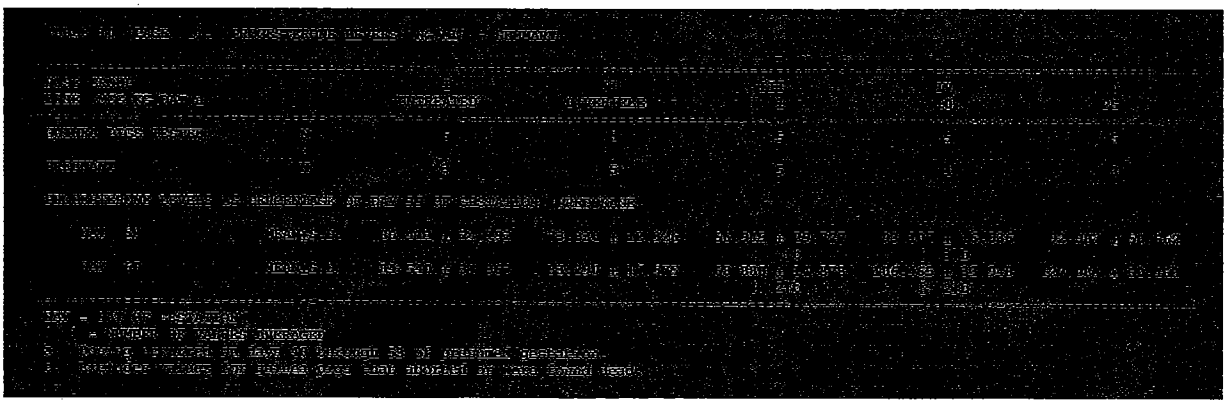
	Assigned Group	Untreated	Vehicle	1.0 µg/kg/day	10 µg/kg/day	25 µg/kg/day
Aborted	**Main**	0	0	0	2	5
	Satellite	0	0	2	2	0
Total	**Main plus Satellite**	0	0	2	4	5

Litter size and live fetuses: There were 21 (87.5%), 17 (70.8%), 21 (87.5%), 21 (87.5%) and 23 (95.8%) pregnant guinea pigs in the untreated, 0 (vehicle), 1, 10 and 25 µg/kg dose groups, respectively. No significant differences in litter size, number of live or dead fetuses, or number of resorptions were observed between the controls and the treatment group animals. Pregnancy and cesarean section observations in different groups are summarized in the Table below.

Observation	Control (Untreated) 1	Control (Vehicle) 2	1 µg/kg 3	10 µg/kg 4	25 µg/kg 5
Pregnant	21 (87.5%)	17 (70.8%)	21 (87.5%)	21 (87.5%)	23 (95.8%)
Found dead	0 (0%)	1 (5.9%)	2 (9.5%)	0 (0%)	3 (13%)
Moribund sacrificed	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)
Aborted and sacrificed	0 (0%)	0 (0%)	0 (0%)	2 (9.5%)	5 (21.7%)
Pregnant and cesarean-sectioned on GD 60	21	16	19	19	14
Animals with viable fetuses	21 (100%)	16 (100%)	19 (100%)	19 (100%)	14 (100%)
Corpora lutea	6.0 ± 1.5	5.6 ± 1.5	6.0 ± 2.0	5.4 ± 2.1	5.4 ± 1.7
Implantations	5.0 ± 1.4	4.9 ± 1.6	5.2 ± 2.2	4.5 ± 2.2	4.2 ± 2.0
Litter sizes	5.0 ± 1.3	4.8 ± 1.5	5.0 ± 2.1	4.3 ± 2.2	3.8 ± 2.0
Live fetuses	5.0 ± 1.3	4.8 ± 1.5	5.0 ± 2.1	4.3 ± 2.2	3.8 ± 1.9
Dead fetuses	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
Resorptions	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.7	0.4 ± 0.6
Early resorptions	0.0 ± 0.2	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 0.5	0.1 ± 0.4
Late resorptions	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.2	0.2 ± 0.4

N=24; **Mean ± S.D**

Serum Progesterone Concentrations: Serum progesterone concentrations of the satellite animals (5 animals/group) were determined on gestation days 39, 53 and 60. Progesterone levels of individual animals were variable; however, no significant differences were observed among different groups. The mean progesterone levels of different groups of animals on gestation days 53 and 60 are shown in the Table below.



The serum progesterone levels (ng/ml) of individual animals from different groups are shown in the Table below.

Treatment Group	Gestation Day-38	Gestation Day-53	Gestation Day-60
Control (untreated)			
#7075 P	142.3	128.10	95.103
#2374 NP	0.779	0.354	2.382
#273 P	157.20	95.30	98.478
#5038 P	103.50	112.40	128.50
#7376 NP	4.812	1.170	6.442
Control (vehicle)			
#8600 P	92.20	84.90	93.33
#2330 P	145.70	113.90	139.30
#8797 P	147.30	106.20	133.40
602 P	139.20	82.40	87.521
#9038 P	136.40	92.20	90.283
1 µg/kg			
#8305 P	30.888	Found dead on GD52	--
#556 P	150.80	89.00	65.967 (aborted on GD57)
#1304 P	128.40	124.90	101.948
#582 P	146.00	40.80	85.202
#7876 P	36.472	15.885	22.339 (aborted on GD55)
10 µg/kg			
#9999 P	100.10	110.50	130.60
#8639 P	153.40	138.40	126.50
#8516 NP	1.001	0.796	2.683
#9030 P	25.541	20.104	(aborted on GD54)
#376 P	40.887	39.756	(aborted on GD 48)
25 µg/kg			
#7271 P	151.10	66.675	1.37.10
#3070 P	42.861	62.270	84.384
#6359 NP	1.591	0.751	0.628
#2378 P	146.30	131.011	137.000
#8046 NP	2.888	1.581	2.545

P = pregnant; NP = non-pregnant

Summary:

To examine the abortifacient and/or resorption potential of RU-0211 in guinea pigs, the drug was administered to pregnant animals on gestation days 40 through 53 at oral (capsule) doses of 0 (untreated), 0 (vehicle control) 1, 10 and 25 µg/kg. Maternal animals receiving the 25 µg /kg dose, showed abnormal clinical signs. The mean body weights of the high dose animals were slightly lower than that of controls during the dosing and gestation periods. Administration of RU-0211 to the pregnant guinea pigs was associated with dose-dependent abortions. In the main study groups, 1, 2 and 4 animals were found dead or sacrificed in moribund condition in the vehicle control, 1 and 25 µg /kg groups, respectively. In the main study groups, two (of 21) animals from the 10 µg /kg group, and 5 (of 23) animals from the 25 µg /kg main study group had abortions. In addition, 2 satellite group animals (of 5), each from the 1 µg /kg and 10 µg /kg had abortions. There were no abortions in the untreated or vehicle control groups. Thus, RU-0211 caused dose-dependent abortions in guinea pigs.

Study title: A study to Evaluate the Abortifacient Effect of RU-0211 in Rhesus Monkeys by Oral Gavage Administration

Key study findings: RU-0211, at oral doses up to 30 µg/kg, did not cause dose-dependent abortions in rhesus monkeys when administered on gestation days 110 to 130. However, the dose selection for the study was not appropriate.

Study no.: SBL99-46
Conducting laboratory and location:

Date of study initiation: February 09, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: RU-0211, Lot #12
Formulation/vehicle: RU-0211 was dissolved in medium chain fatty acid triglyceride (MCT;) at concentrations of 30, 100 and 300 µg/ml. Control animals were administered MCT. The drug substance was stable in MCT solution for up to (of the initial concentration).

Methods:

Doses: RU-0211 was administered by oral gavage at 0, 10 and 30 µg/kg (0.1 ml/kg) doses.

Study design: The study was conducted in pregnant rhesus monkeys of about 5 to 11 years old (body weights- 3.72 to 7.42 kg at mating). The drug was administered by oral gavage at 0, 10 and 30 µg/kg doses via a nasogastric tube (internal diameter 1 mm) on gestation days 110 through 130 (total 21 days). There were 10, 10 and 11 pregnant animals in the 0, 10 and 30 mg/kg/day groups, respectively. The middle day of the 5-day mating period was designated the day 0 of gestation (GD0). Pregnancy was diagnosed by ultrasound between gestation days 25 and 30. Females judged as non-pregnant were mated at the next menstrual cycle up to a maximum of 3 times, with the exception of 4 females that were mated 4 times. The animals were randomly assigned to three groups based on their body weights on gestation day 2.

The sponsor stated that the doses for the study were selected on the basis of reproduction studies in rats, in which 0.2 mg/kg was identified as the NOEL, and as the rhesus monkeys are 3 to 20 times more sensitive than rats to the abortifacient effects of a similar class of compounds, 30 µg/kg (0.03 mg/kg) was selected as the high dose for the study.

The animals were observed twice daily during the dosing period, and once daily during the non-dosing period for clinical signs and mortality. Embryo/fetal viability was monitored by ultrasound on gestation days 40, 50, 70, 90, 108, 115, 122, 129, 136 and 143 (± 1 day). Food consumption and body weights of the animals were recorded at regular intervals. Serum progesterone levels of all monkeys were determined on gestation days 109, 129 and 149 (except animal # 141, whose progesterone levels were determined on gestation day 141). Two prematurely delivered neonates were examined for viability and general health conditions, including external abnormalities.

Pregnancy was terminated on gestation day 150 or 151 by cesarean section, and each fetus was subjected to standard teratological evaluation.

Results:

Clinical signs: No treatment-related abnormal clinical signs were observed in any group. One monkey from the 30 µg/kg group (#10205) and another monkey from the 10 µg/kg group (#10107) had early delivery on day 149 of gestation. One monkey from the 10 µg/kg group had an abortion on day 141 of gestation. There was no abortion in the 30 µg/kg group.

Body Weights and Food Consumption: The mean body weight of the control animals on gestation day 2 was 5.11±1.10 kg. No significant changes in body weight and food consumption were observed between the control and treatment groups.

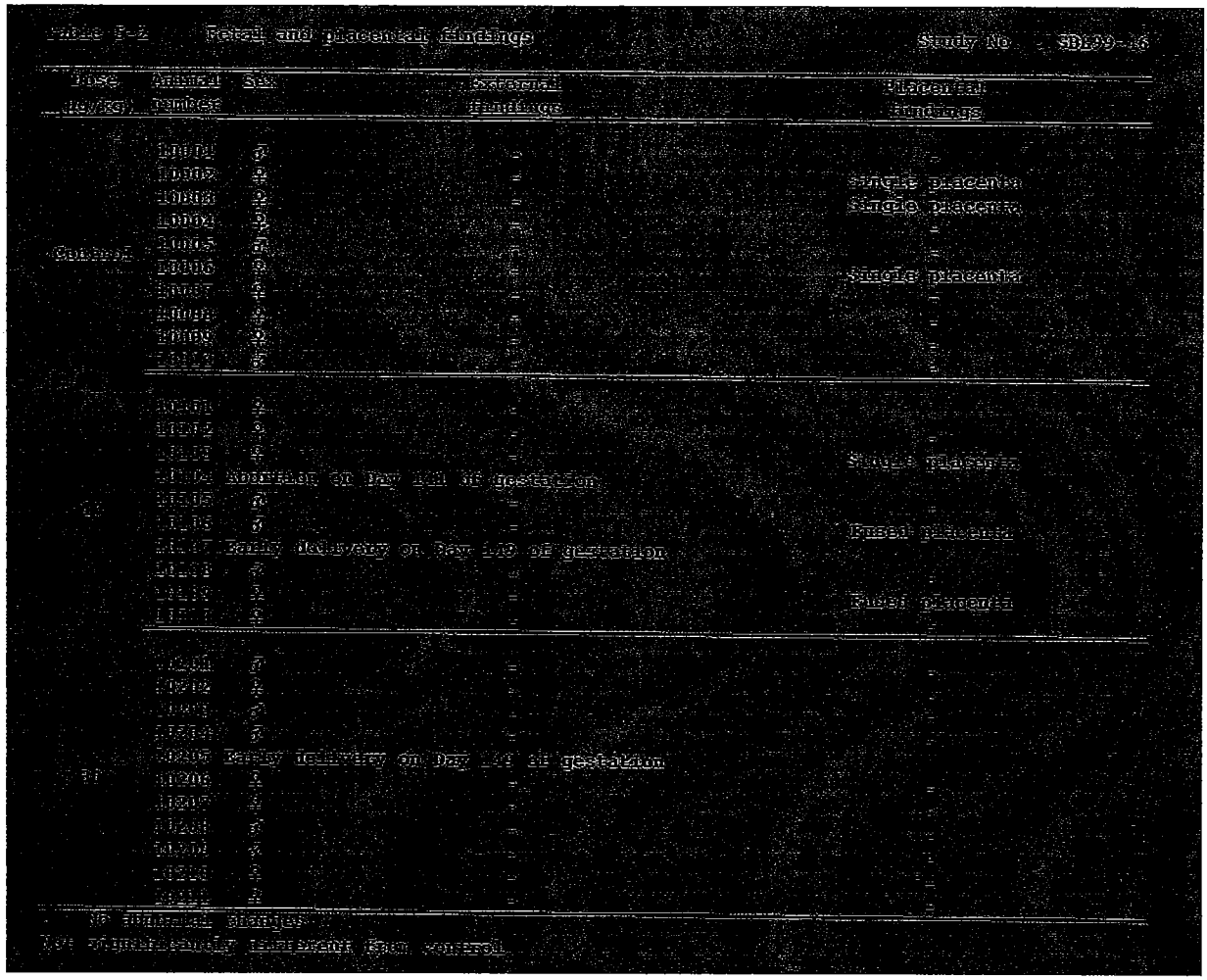
Serum Progesterone Concentrations: Serum progesterone concentrations of the animals were determined on gestation days 109, 129 and 149. No significant differences in serum progesterone concentrations were observed between the control and the treatment groups. Mean serum progesterone levels (ng/ml) of the control and treatment group animals are shown in the Table below.

Treatment Group	Gestation Day-109	Gestation Day-129	Gestation Day-149
Control	5.87 ± 3.09	5.49 ± 2.64	7.55 ± 3.48
10 µg/kg	5.72 ± 2.62	4.61 ± 1.80	6.49 ± 3.18
30 µg/kg	4.92 ± 1.78	4.27 ± 1.65	6.10 ± 2.50

Premature Delivery: Early delivery on Day 149 of gestation was observed in one animal (#10205) in the 30 mg/kg group and in one animal (#10107) in the 10 mg/kg group. These neonates were delivered naturally and alive. No lactational abnormalities were observed in these monkeys. No abnormalities were observed in the external features or general health condition of the neonates. The incidences of abortion and early delivery in different groups are summarized in the Table below.

	0 µg/kg	10 µg/kg	30 µg/kg
No. of pregnant monkeys	10	10	11
Abortion n (%)	0 (0%)	1 (10%)	0 (0%)
Early delivery n (%)	0 (0%)	1 (10%)	1 (9.1%)

Fetuses: No external abnormalities were observed in any fetus, and no significant differences in fetal body weight and placental weight were observed between the control and the treatment groups. Fetal and placental findings in different groups are shown in the Table below.



Summary:

To examine the abortifacient potential of RU-0211 in rhesus monkeys, the drug was administered to pregnant animals on gestation days 110 through 130 at oral (gavage) doses of 0, 10 and 30 µg/kg/day. The dose selection for the study was based on the NOEL from reproductive toxicity studies in rats, and was not appropriate. Early delivery (on gestation day 149) was observed in one animal receiving the 30 µg /kg dose and another animal receiving the 10 µg/kg dose (historical control data from the conducting laboratory has shown natural deliveries between gestation day 144 and gestation day 171). The deliveries were normal, and no abnormalities were observed in these two neonates. One monkey from the 10 µg/kg group had an abortion on gestation day 141. No treatment-related changes in fetal external examinations, fetal body weights, placental examinations or placental weights were observed in any group.

Thus, RU-0211 did not cause a dose-dependent abortion in rhesus monkeys when administered at oral doses up to 30 µg/kg. However, the dose selection for this study was based on NOEL in rats, and was not appropriate.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

Tables and Figures are incorporated in appropriate sections of the review.

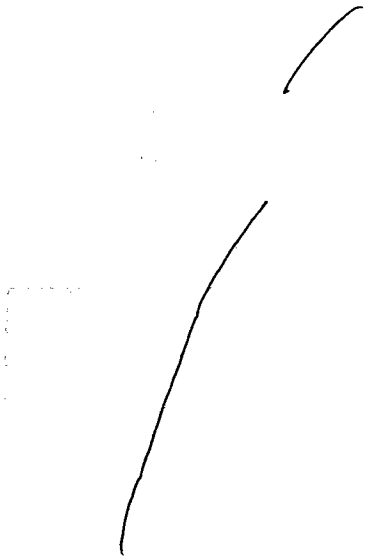
2.6.7 TOXICOLOGY TABULATED SUMMARY

N/A

LABELING:

The draft labeling of ETRVA (Lubiprostone) capsules does not conform to the format specified under 21 CFR, Subpart B – Labeling Requirements for Prescription Drugs. The following changes in the labeling should be incorporated.

Sponsor's Version:



Evaluation:

This section is not in accordance with the 21 CFR. The heading should be changed to 'Carcinogenesis, Mutagenesis, Impairment of Fertility' and the 'Impairment of Fertility' section should be combined with this section of the labeling. In the 2-year oral carcinogenicity study in mice, there was significant increase in the incidence of Harderian gland carcinoma in the female animals at a dose of 500 µg/kg/day. In the 2-year oral carcinogenicity study in rats, there were increased incidences of squamous cell papilloma and papilloma plus carcinoma in the nonglandular stomach of the male animals. There were also higher incidences of histiocytic sarcoma and benign interstitial cell tumor of the testes of male rats receiving the 400 µg/kg/day dose. In female rats, treatment with RU-0211 produced hepatocellular adenoma at a dose of 400 µg/kg. These findings should be included in the labeling.

Proposed Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Two 2-year carcinogenicity studies (one in Crl:B6C3F1 mice and one in Sprague-Dawley rats) were conducted with RU-0211. In the 2-year carcinogenicity study in mice, RU-0211doses of 25, 75, 200 and 500 µg/kg/day (about 2, 6, 17 and 42 times the human dose, respectively, based on body surface area) were used. In the 2-year rat carcinogenicity study, RU-0211 doses of 20, 100

and 400 µg/kg/day (about 3, 17 and 68 times the human dose, respectively, based on body surface area) were used. In the mouse carcinogenicity study, there was no significant increase in any tumor incidences.

. In female rats, treatment with RU-0211 produced hepatocellular adenoma.

RU-0211, at oral doses up to 1000 µg/kg/day, had no effect on the fertility and reproductive performance of male and female rats. The 1000 µg/kg/day dose in rats is about 166 times the recommended human dose of 48 µg/day, based on the body surface area.

Evaluation: RU-0211 was abortifacient in guinea pigs when administered to the pregnant animals. Thus, RU-0211 should be classified under pregnancy category C

Proposed Version:
Pregnancy Category C: Teratology studies with RU-0211have been conducted in rats at oral doses up to 2mg/kg/day (about 332 times the proposed human dose, based on body surface area), and in rabbits at oral doses up to 0.10 mg/kg/day (about 33 times the proposed human dose, based on body surface area). RU-0211 was not teratogenic in rats and rabbits.
in guinea pigs, RU-0211 caused
(about 2 and 6 times the human dose, respectively, based on the body surface area).

Nursing Mothers:
Sponsor's version:

Evaluation: This section does not conform to the format specified under 21 CFR, Subpart B.

Proposed version:
It is not known whether is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from

— a decision should be made whether to discontinue —

OVERDOSAGE:

Sponsor's version:
There have been two confirmed reports of overdosage with — The first report involved a 3-year old child who accidentally ingested 7 to 8 capsules of 24 mcg — and fully recovered. The second report was a study subject who self-administered a total of 96 mcg — per day for 8 days. The subject experienced no adverse events during this time. Additionally, in a definitive phase I cardiac repolarization study, 51 subjects were dosed with a single oral administration of 144 mcg — that is six times the normal single administration dose. Thirty nine (39) of the 51 subjects experienced an adverse event. The adverse events reported in >1% of this group included: abdominal pain (5.9%), abdominal pain upper (2.0%), diarrhea (25.5%), dry mouth (2.0%), loose or watery stools (13.7%), nausea (45.1%), retching (7.8%), stomach discomfort (3.9%), vomiting (27.5%), asthenia (2.0%), chest discomfort (2.0%), flushing or hot flush (5.9%), hyperhydrosis (2.0%), pallor (3.9%), anorexia (2.0%), dizziness (17.6%), headache (11.8%), syncope (3.9%), vasovagal episode (2.0%), dyspnea (3.9%) and skin irritation (2.0%).

Evaluation: No changes recommended.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

RU-0211, a prostaglandin E1 analog, is a specific activator of CIC-2 chloride channels, which is involved in the secretion of fluids in the gastrointestinal tract. It caused a dose-dependent increase in intestinal fluid secretion in rats. The main metabolite of RU-0211, 15-hydroxy-RU-0211 (M3) also caused a dose-dependent increase in the intestinal fluid secretion in rats, with a potency similar to that of the parent compound. RU-0211 at oral doses of 1 to 100 μg/kg, caused an increase in intestinal transit in morphine-treated mice. Thus, as RU-0211 caused an increase in intestinal fluid secretion and intestinal transit in animals, it may be useful in the treatment of patients with constipation.

Under the current NDA, the sponsor submitted the following preclinical studies with RU-0211: Pharmacology; Absorption, Distribution, Metabolism and Excretion (ADME) studies in rats, mice and dogs; Toxicology- single dose oral toxicity studies in rats and dogs, repeated dose toxicity studies: 2-week oral toxicity study in rats, 4-week oral toxicity study in rats, 26-week oral toxicity study in rats, 13-week oral toxicity study in mice, 2-week oral toxicity study in dogs, 4-week oral toxicity study in dogs, 39-week oral toxicity study in dogs; Genotoxicity- Bacterial reverse mutation (Ames assay) assay, chromosomal aberration assay in Chinese hamster lung (CHL) cells, mouse lymphoma cell forward gene mutation assay, mouse bone marrow micronucleus assay; Carcinogenicity- 104-week oral carcinogenicity study in mice, 104-week oral carcinogenicity study in rats; Reproductive and Developmental Toxicity- Oral Segment I fertility

and general reproductive performance study in rats, oral Segment II teratogenicity study in rats, oral Segment II teratogenicity study in rabbits, oral Segment III pre- and post- natal development study in rats; Local Tolerance- antigenicity study in guinea pigs; Special Toxicity Studies- abortifacient study in rhesus monkeys, abortifacient study in guinea pigs.

RU-0211 was rapidly absorbed in rats, mice and dogs following oral administration. Following oral administration of single doses of 25, 50 and 100 µg/kg doses to rats, maximum plasma concentrations were reached in 0.5 to 0.75 hr, and the terminal elimination half-lives ($t_{1/2}$) ranged from 2 to 6 hours. In mice, following oral administration of 25 and 50 µg/kg doses, the T_{max} was 10 to 15 minutes, and the $t_{1/2}$ was about 3 hours. In contrast to rats, in dogs the T_{max} for the total radioactivity was 3 hours following oral administration, and the $t_{1/2}$ was longer than in rats. In rats, there were no signs of accumulation of the drug in any specific tissue. In rats, dogs and mouse, RU-0211 was metabolized rapidly, and several metabolic peaks were detected in the plasma samples. Among them, 15-hydroxy-RU-0211 was the least polar metabolite, and was found be biologically active, with a potency similar to the parent compound. No other metabolite showed the pharmacological activity. Microsomal CYP enzymes had no major role in the metabolism of RU-0211. The parent compound was detected in plasma samples for up to 2 hours after administration. Following oral administration in mice, dogs, rabbits and monkeys, renal excretion was the predominant pathway for elimination of the drug. In rats, following oral administration, fecal excretion was predominant, while following i.v. administration, renal route was the predominant pathway of elimination. In humans, RU-0211 was not detected in the plasma, urine or feces following oral administration of a dose that is 3-fold higher than the proposed clinical dose of 48 µg/day. The main metabolite, M3 was detectable in humans. RU-0211 was highly bound to human plasma proteins (94.6%). Following oral administration of a radiolabeled dose, the radioactivity was primarily eliminated via kidney.

Single and repeat dose toxicology studies with RU-0211 were conducted in rats, mice and dogs. In the acute toxicity study in rats, the minimal lethal dose was 60 mg/kg in males and 30 mg/kg in females. The clinical signs observed in rats included decreased locomotor activity, lacrimation, loose stool and dyspnea. In dogs, single oral doses up to 40 mg/kg was non-lethal, and decreased locomotor activity, loose stool/diarrhea, vomiting, lacrimation, salivation and pale buccal mucosa were observed in males and females.

In a 2 –week oral-gavage toxicity study with RU-0211 in rats, the drug was administered at doses of 0.008, 0.04, 0.2 and 1 and 5 mg/kg. The 5 mg/kg dose was a lethal dose. Loose stool and diarrhea were observed at 1 and 5 mg/kg doses. The target organs of toxicity were the liver (clear cell changes in the hepatocytes), thymus (atrophy), spleen (decreased cellularity of the white pulp), mesenteric lymph nodes (blood resorption) and bone and bone marrow (fibrous osteodystrophy and decreased cellularity of the bone marrow). The no effect dose was 0.04 mg/kg.

In the 4-week oral-gavage toxicity study with RU-0211 in rats, the drug was administered at oral doses of 0.04, 0.2 and 1 mg/kg to male and female animals. Loose stool and abdominal distention were observed in both males and females receiving the high dose. Mineralization of the germinal center of the ileum was observed in males and females receiving the high dose. Enlargement and increased weight of the adrenals was observed in males and females receiving the 1 mg/kg dose, and histopathological

examination revealed hyperplasia of the zona fasciculata in females at all doses and in the high dose males. The target organ of toxicity was the adrenal gland and the gastrointestinal tract, and the no effect dose was 0.04 mg/kg in the females and 0.2 mg/kg in the males.

In a 26-week oral toxicity study in rats, groups of animals received RU-0211 at doses of 0, 0.016, 0.08, and 0.4 mg/kg/day. The dose of 0.08 mg/kg/day could be considered a tolerated dose. Salivation immediately after dosing was observed in all male and female treatment groups. Final body weights (92% of control) and body weight gains (87.6% of control) were slightly suppressed for male rats at 0.4 mg/kg/day. Water consumption was increased for all male treatment groups and female rats at 0.4 mg/kg/day. The stomach was the target organ of toxicity. Proliferation of epithelial basal cells in the limiting ridge of the stomach was observed for all male and female treatment groups.

In the 13-week dose range finding study with RU-0211 in mice, groups of animals received oral doses of 0, 0.01, 0.1 and 1, and 5 mg/kg/day of the drug. Loose stool and/or diarrhea were observed in both sexes receiving 1 and 5 mg/kg/day doses. Abdominal distention and decreased locomotor activity were observed in males and females receiving the high dose. Mild thickening at the limiting ridge of the stomach was observed in both males and females receiving 0.1 mg/kg and higher doses; histopathological examination revealed edema and acanthosis in the stomach of both males and females at 1 and 5 mg/kg/day doses. High dose males (4 of 10) had swelling of the cortical cells of the adrenal glands. The target organs of toxicity were the stomach and the adrenal gland. The 1 mg/kg dose can be considered as the maximum tolerated dose as the changes observed at this dose were related to the pharmacological effects of the compound and only mild changes in the stomach was observed.

In the 2-week oral (capsule) toxicity study with RU-0211 in dogs, the drug was administered at 0.04, 0.2 and 1 mg/kg doses. Diarrhea, loose stool and vomiting were observed in the treatment group animals in a dose-dependent manner. Decreased locomotor activity and lacrimation were observed at the 1 mg/kg dose. No target organ of toxicity was identified, and the no effect dose was not established. The high dose (1 mg/kg) was the tolerated dose.

In the 4-week oral gavage toxicity study with RU-0211 in dogs, the drug was administered to male and female animals at oral doses of 0.01, 0.07 and 0.5 mg/kg/day. Loose stool, diarrhea and watery stool were observed at all doses in both males and females and the incidences were increased with increasing doses. Vomiting, salivation and lacrimation were observed in both sexes at 0.07 mg/kg and higher doses. There were dose dependent decreases in sodium and chloride concentrations in the urine of both males and females. This could be the secondary to the loss of the electrolytes due to loose stool/diarrhea. Slight to mild hyperplasia of the zona glomerulosa was observed in both males and females receiving the high dose, being prominent in females. The target organ of toxicity was the adrenal gland and the gastrointestinal tract and the no effect dose was 0.01 mg/kg in both males and females.

In the 39-week oral toxicity study in Beagle dogs, groups of animals were administered oral doses of 0, 0.002, 0.01 and 0.05 mg/kg of RU-0211. Dose-related increases in vomiting of bubbly gastric juice,

diarrhea and loose stool or watery stool were observed in both males and females at 0.01 and 0.05 mg/kg/day doses. The high dose males had decreased urinary excretion of sodium and the high dose females had decreased urinary excretion of sodium and chloride. Males receiving the high dose had focal hepatocytic necrosis (1 of 4), atrophy of the seminiferous tubule (2 of 4) and cellular infiltration of lymphoid/plasma cells in bilateral testis (1 of 4), and pyelitis in the kidney was observed in both males and females receiving the high dose (1 of 4 each). The no effect dose was 0.01 mg/kg/day and the target organs of toxicity was the testis in the males and kidney in both males and females.

The genotoxic potential of RU-0211 was evaluated using the bacterial reverse mutation assay (Ames assay), the *in vitro* chromosome aberration assay in Chinese hamster lung (CHL) cells, the *in vitro* mouse lymphoma cell forward gene mutation assay and the *in vivo* mouse bone marrow micronucleus assay. RU-0211 had no genotoxic potential in any of these assays.

The carcinogenic potential of RU-0211 was assessed in a 104-week oral carcinogenicity study in mice and a 104-week oral carcinogenicity study in rats.
In the 104-week carcinogenicity study in mice, RU-0211 doses of 25, 75, 200 and 500 µg/kg/day were used. The dose selection for the mouse carcinogenicity study was based on the MTD and was concurred by the CDER Executive CAC. There was an increased incidence of Harderian gland carcinoma [control, 0/55 (0%); 25 µg/kg, 0/55 (0%); 75 µg/kg, 1/55 (1.8%); 200 µg/kg, 2/55 (3.6%); 500 µg/kg, 2/55 (3.6%)], as well as adenoma plus carcinoma [control, 2/55 (3.6%); 25 µg/kg, 2/55 (3.6%); 75 µg/kg, 3/55 (5.5%); 200 µg/kg, 3/55 (5.5%); 500 µg/kg, 7/55 (12.7%)] in female mice treated with RU-0211. However, the incidences were not statistically significant for common tumor types ($p>0.005$, trend test).

In the 104-week rat carcinogenicity study, the doses of RU-0211 used were 20, 100 and 400 µg/kg/day. The dose selection for the rat carcinogenicity study was based on the MTD and was concurred by the CDER Executive CAC. In male rats, there was an increased incidence of squamous cell papilloma [control, 1/65 (1.5%); low dose, 1/65 (1.5%), mid dose, 5/65 (7.7%), high dose, 6/64 (9.4%)], as well as squamous cell papilloma plus carcinoma [control, 2/65 (3.1%); low dose, 1/65 (1.5%), mid dose, 6/65 (9.2%), high dose, 7/64 (10.9%)] of the non-glandular stomach. However, the incidences for these tumors were not statistically significant for common tumor types ($p>0.005$, trend test). There was a significant increase in the incidences of interstitial cell adenoma of the testes [control, 2/65 (3.1%); low dose, 4/65 (6.2%), mid dose, 1/65 (1.5%), high dose, 10/65 (15.4%); $p=0.0006$, trend test] in male rats. In female rats, treatment with RU-0211 produced hepatocellular adenoma [control, 0/65 (0%); low dose, 0/65 (0%), mid dose, 1/65 (1.5%), high dose, 5/65 (7.7%); $p=0.0031$, trend test].

In the oral Segment I fertility and general reproductive performance study with RU-0211 in rats, male and female animals received 0.04, 0.2 and 1.0 mg/kg/day doses of the drug. RU-0211 had no effects on the fertility and reproductive performance of male and female animals at doses up to 0.2 mg/kg/day. There was a decrease in the number of implantation sites and live embryos at a dose of 1.0 mg/kg/day. However, this dose had toxic effects on the animals (decreased body weight, loose stool).
In the oral Segment II teratogenicity study with RU-0211 in rats, pregnant animals were treated with RU-0211 at 0, 0.02, 0.2 and 2.0 mg/kg/day doses on gestation day 6 through 17. RU-0211 was not teratogenic in rats at oral doses up to 2.0 mg/kg/day.

In the oral Segment III teratogenicity study in rabbits, RU-0211 was administered to the pregnant animals at 0, 0.01, 0.03 and 0.10 mg/kg/day doses on gestation days 7 through 20. RU-0211 was not teratogenic in rabbits at oral doses up to 0.10 mg/kg/day.

In the Segment III pre- and post-natal developmental toxicity study with RU-0211 in rats, pregnant animals were treated with 0, 0.02, 0.2 and 1.0 mg/kg/day doses. Treatment with RU-0211 had no significant effect on the pre- and post- natal development of the F1 animals at doses up to 0.2 mg/kg. High dose F0 females had decreased body weight gains during the gestation period, and half of the dams had total litter death during parturition. The viabilities of pups and mean pup weights from dams receiving the 1.0 mg/kg/day dose were lower than that of controls.

The abortifacient potential of RU-0211 was examined in guinea pigs and Rhesus monkeys following oral administration of the drug to pregnant animals. In guinea pigs, the abortifacient potential of RU-0211 was examined after oral administration of 1, 10 and 25 µg/kg doses on gestation days 40 through 53. Abnormal clinical signs and mortalities were observed at the 25 µg/kg dose. Two (of 21) animals receiving the 10 µg/kg dose and 5 (of 23) animals receiving the 25 µg/kg dose had abortions. In addition, from the satellite groups (used for serum progesterone determinations), 2 animals each (of 5) from the 1 and 10 µg/kg had abortions. Thus, treatment with RU-0211 was associated with dose-dependent abortions in guinea pigs.

To examine the abortifacient potential of RU-0211 in rhesus monkeys, the drug was administered to pregnant animals (0, 10 and 30 µg/kg, orally) on gestation days 110 through 130. The dose selection for the monkey abortifacient study was based on the NOEL from reproductive toxicity studies in rats, and was not appropriate. One monkey from the 10 µg/kg group had an abortion. In addition, one animal each from the 10 mg/kg and 30 mg/kg had early deliveries.

Conclusions:

RU-0211 is a prostaglandin analog and is a specific activator of CIC-2 chloride channels, which is involved in the secretion of fluids in the gastrointestinal tract. The sponsor submitted NDA 21-908 for RU-0211 for the treatment of adult subjects with chronic idiopathic constipation [redacted]. In human intestinal T_{84} cells, RU-0211 caused a concentration-dependent increase in chloride currents, and in rats, it caused a dose-dependent increase in intestinal fluid secretion. The metabolite of RU-0211, 15-hydroxy-RU-0211 also caused a dose-dependent increase in intestinal fluid secretion in rats, with a potency similar to the parent compound. RU-0211 also decreased the intestinal transit time in morphine-treated rats. Thus, RU-0211 may be useful in relieving the symptoms of chronic constipation.

The sponsor conducted adequate non-clinical studies with RU-0211 to determine the safety of the drug by the proposed route of administration. In safety pharmacology studies with RU-0211, it had no effects on the central nervous system, respiration or cardiovascular functions. In toxicology studies in rats and mice, proliferation of the epithelial basal cells in the limiting ridge of the stomach was observed in animals receiving repeated doses of the drug. Loose stool and/or diarrhea were observed in all species examined. In repeated dose toxicity studies, hyperplasia of the zona glomerulosa of the adrenal gland was observed in rats, mice and dogs. Thus, the common target organs of toxicity were the gastrointestinal tract and the adrenal gland. In the chronic toxicity study in dogs, the kidney was identified as the target organ of toxicity in both males and

females. RU-0211 was not genotoxic in a battery of genotoxicity assays. It had no effects on the reproductive performance of male and female rats, and was not teratogenic in rats and rabbits. RU-0211 was abortifacient in guinea pigs when administered to the pregnant animals.

Unresolved toxicology issues (if any): None

Recommendations:

The sponsor conducted adequate preclinical studies with RU-0211 to determine the safety of the drug, and the sponsor's proposed dose appears to be safe for the proposed indication. Thus, from a preclinical standpoint, the NDA application is approvable. However, RU-0211 was abortifacient in guinea pigs. Thus it may endanger pregnancy when administered to pregnant women. So, the drug should not be taken by pregnant women. This should be clearly stated in the labeling of RU-0211,

Suggested labeling: See the labeling part of the review.

Sushanta Chakder, Ph. D. Date
Pharmacologist, HFD-180

Comments:

Jasti B. Choudary, Ph.D., B. V. Sc. Date
Supervisory Pharmacologist, HFD-180

cc.
NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Chakder

HFD-180/Dr. Choudary

HFD-102/Dr Jacobs

HFD- 048/Dr. Viswanathan

R/D Init.: J. Choudary 12/09/05

APPENDIX/ATTACHMENTS:

APPENDIX 1: Microscopic Observations in Male and Female Mice.

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
*** TOP OF LIST ***											
PITUITARY (PI)	NUMBER EXAMINED:	49	51	51	48	51	53	52	52	51	53
	NOT REMARKABLE:	49	44	51	44	49	28	19	29	26	25
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	2	0	0	0
--B-ADENOMA		0	0	0	0	0	13	14	10	13	15
--HYPERPLASIA, FOCAL		0	3	0	0	1	12	14	14	12	11
--CYST(S)		0	5	0	4	1	0	2	0	0	0
--ANGIECTASIS		0	0	0	0	0	1	3	5	3	2
--FOCAL PIGMENT (OLD HEMORRHAGE)		0	0	0	0	0	0	0	0	0	1
--HEMATOCYST		0	0	0	0	0	0	1	0	0	0
BRAIN (BR)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	30	24	31	25	25	31	25	25	37	20
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	1	2	2	0	0
--MINERALIZED FOCI (BACKGROUND)		23	31	24	30	30	22	26	30	16	34
--VENTRICLE, DILATATION		1	0	0	0	0	0	0	0	1	0
--LYMPHOCYTIC PERIVASCULAR CUFF, MENINGES		0	0	0	0	1	3	1	1	1	2
--VENTRAL DISTORTION (PITUITARY TUMOR)		0	0	0	0	0	1	0	0	2	1
--LYMPHOCYTIC INFILTRATE, CHOROID PLEXUS		0	0	0	0	0	0	1	0	0	0
CORD, CERVICAL (CS)	NUMBER EXAMINED:	55	54	55	55	55	55	54	55	55	55
	NOT REMARKABLE:	55	54	55	55	54	54	52	54	55	55
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	0	0
--LYMPHOCYTIC PERIVASCULAR CUFF, MENINGES		0	0	0	0	1	1	1	0	0	0
--DEGENERATION, CHRONIC INFLAMMATION		0	0	0	0	0	0	0	1	0	0
CORD, LUMBAR (LC)	NUMBER EXAMINED:	55	54	55	55	55	55	54	53	55	55
	NOT REMARKABLE:	54	54	55	55	55	53	53	51	54	53
--DEMYELINATION		0	0	0	0	0	0	0	0	0	1
--LYMPHOCYTIC PERIVASCULAR CUFF, MENINGES		1	0	0	0	0	2	1	2	1	1

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ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
CORD, THORACIC (TC)	NUMBER EXAMINED:	55	54	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	55	54	55	55	55	55	54	54	55	54
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--LYMPHOCYTIC PERIVASCULAR CUFF, MENINGES		0	0	0	0	0	0	0	1	0	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	54	54	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	1	5	8	9	2	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0	0	4	3	2	0
--B-ADENOMA		3	1	2	2	2	1	1	0	0	0
--B-ADENOMA, SUBCAPSULAR CELL		0	1	1	0	2	0	0	0	0	0
--N-LEIOMYOSARCOMA, METASTATIC		0	0	1	0	1	0	0	0	0	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--I-SARCOMA, INVASIVE		0	0	0	0	0	0	0	0	0	1
--HYPERPLASIA, SUBCAPSULAR CELL (BACKGROUND)		51	47	46	44	50	53	53	54	54	53
--CONGESTION/HEMORRHAGE, CORTICOMEDULLARY JUNCTION (BACKGROUND)		0	0	0	0	0	35	22	27	28	31
--VACUOLIZATION, CORTICOMEDULLARY JUNCTION (BACKGROUND)		0	0	0	0	0	11	7	8	7	5
--PSEUDOAMYLOID, CORTICOMEDULLARY JUNCTION (BACKGROUND)		6	2	8	4	6	46	44	51	42	48
--LIPOFUSCINOSIS, CORTICOMEDULLARY JUNCTION		3	5	1	0	2	29	29	36	31	35
--HEMATOPOIESIS		0	0	0	0	0	0	0	0	1	0
--MONONUCLEAR CELL INFILTRATE		0	0	0	0	1	0	0	0	0	0
--HYPERPLASIA, FOCAL		3	3	1	3	1	1	0	2	2	1
--HYPERTROPHY, FOCAL		18	18	14	11	21	4	4	2	2	1
--LIPIDOSIS, FOCAL		4	1	0	0	0	0	0	1	0	1
--BALLOONING DEGENERATION, FOCAL		0	0	0	0	0	1	0	0	0	0
--CYST(S)		0	0	0	0	0	0	0	0	2	1

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	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	53	54	54	54	55	52	55	55	52	55
	NOT REMARKABLE:	53	54	54	54	55	52	53	55	51	55
--B-PHEOCHROMOCYTOMA		0	0	0	0	0	0	0	0	1	0
--B-PHEOCHROMOCYTOMA, COMPLEX		0	0	0	0	0	0	1	0	0	0
--M-MALIGNANT PHEOCHROMOCYTOMA		0	0	0	0	0	0	1	0	0	0
THYROID (TY)	NUMBER EXAMINED:	53	54	54	55	54	54	53	55	54	55
	NOT REMARKABLE:	41	42	44	45	49	38	40	36	34	43
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	2	0	0	0
--B-FOLLICULAR CELL ADENOMA		3	1	3	2	0	4	2	4	3	3
--M-FOLLICULAR CELL CARCINOMA		1	1	2	0	0	0	0	0	0	1
--HYPERPLASIA, FOLLICULAR CELL		5	3	3	6	3	6	10	9	13	11
--DISTENDED FOLLICLE(S)		2	7	3	2	3	9	3	8	10	1
--CHRONIC VASCULITIS, ADJACENT TISSUE		1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	1	0	0	0	3	3	3	1	1
--MICROABSCESS		0	0	0	0	0	0	0	1	0	0
PARATHYROID (PT)	NUMBER EXAMINED:	42	49	41	41	42	47	41	45	50	47
	NOT REMARKABLE:	42	48	40	41	42	47	40	44	49	47
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	0	0
--CYST		0	1	1	0	0	0	0	0	1	0
--HYPERPLASIA, FOCAL		0	0	0	0	0	0	0	1	0	0
ESOPHAGUS (ES)	NUMBER EXAMINED:	55	54	55	53	55	55	55	55	54	55
	NOT REMARKABLE:	55	53	55	53	54	54	52	55	54	54
--DEGENERATION, MUSCLE		0	1	0	0	1	1	2	0	0	1
--INFLAMMATION, CHRONIC		0	0	0	0	0	0	1	0	0	0

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	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
TRACHEA (TR)	NUMBER EXAMINED:	54	55	53	55	50	54	54	51	50	53
	NOT REMARKABLE:	54	55	53	55	50	54	52	50	50	53
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
--BLOOD-FILLED		0	0	0	0	0	0	1	0	0	0
--DILATED GLAND		0	0	0	0	0	0	1	0	0	0
LUNG (LU)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	27	29	33	26	26	30	26	30	32	32
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	3	2	1	4	10	12	9	6
--B-ADENOMA, BRONCHIOLOALVEOLAR		2	3	2	4	0	0	1	3	2	1
--M-CARCINOMA, BRONCHIOLOALVEOLAR		4	7	3	1	8	1	2	1	2	1
--N-CARCINOMA, HEPATOCELLULAR, METASTATIC		0	2	0	2	0	0	0	0	0	1
--N-CHOLANGIOCARCINOMA, METASTATIC		1	0	0	0	0	0	0	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	1	0	1	1
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--N-CARCINOMA, METASTATIC HARDERIAN GLAND		0	0	0	0	0	0	0	0	1	1
--N-FIBROSARCOMA, BIOMEDIC IMPLANT, METASTATIC		1	0	0	0	0	0	0	0	0	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--N-RENAL TUBULAR CARCINOMA, METASTATIC		0	0	0	1	0	0	0	0	0	0
--TUMOR-RELATED INFLAMMATION		4	1	2	1	4	2	0	0	1	0
--HYPERPLASIA, BRONCHIOLOALVEOLAR		6	1	2	4	3	1	0	2	2	3
--INFLAMMATION, FOCAL		0	1	1	1	3	6	1	1	0	3
--INFLAMMATION, CHRONIC, FOCAL		5	0	1	1	3	0	1	0	2	2
--HEMORRHAGE		0	0	0	0	0	0	1	0	0	0
--CONGESTION, HEMORRHAGE (AGONAL)		6	12	13	4	8	9	10	10	6	5
--INFLAMMATORY FOCI (BACKGROUND)		6	6	1	8	3	6	5	4	4	1
--THROMBI, VENOUS		0	0	0	0	1	0	0	0	0	0
--ACIDOPHILIC HISTIOCYTIC PNEUMONIA		0	1	0	0	2	0	0	0	1	0
--ATELECTASIS		0	0	1	0	0	0	0	0	0	0
--HYPERPLASIA, LYMPHOID		1	0	0	0	0	0	1	1	0	1
--INFLAMMATION, CHRONIC, PLEURA		0	0	0	0	1	0	0	0	0	0

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE----------					---------FEMALE---------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
LUNG (LU)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	27	29	33	26	26	30	26	30	32	32
--CHONDROID METAPLASIA, FOCAL		0	0	0	0	1	0	0	0	0	0
--FOREIGN BODY (HAIR FRAGMENT) REACTION		0	0	0	0	0	1	0	0	0	0
--CHRONIC VASCULITIS WITH INTIMAL PROLIFERATION		0	0	0	0	0	1	0	0	0	0
--HISTIOCYTIC FOCI		0	0	0	0	0	1	0	0	0	0
--FOCAL SUBACUTE PNEUMONITIS		0	0	0	0	0	1	0	0	0	0
--OSSEOUS METAPLASIA		0	0	0	2	0	0	0	0	0	0
--INFLAMMATION, ACUTE, FOCAL		0	0	0	1	0	0	0	0	0	0
HEART (HT)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	45	52	44	47	48	47	46	47	45	42
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	2	1	0	3	3	3	1
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	1	1	0	0	0	0	0	0
--N-CHOLANGIOCARCINOMA, METASTATIC		1	0	0	0	0	0	0	0	0	0
--N-RENAL TUBULAR CARCINOMA, METASTATIC		0	0	0	1	0	0	0	0	0	0
--MYOCARDIAL DEGENERATION/CHRONIC INFLAMMATION/FIBROSIS		7	3	6	2	6	8	4	4	5	11
--DEGENERATION, VASCULAR		0	0	1	0	0	0	0	0	0	0
--MINERALIZATION, FOCAL, MYOCARDIUM		1	0	1	2	0	0	0	1	2	0
--INFLAMMATION, CHRONIC, EPICARDIUM		0	0	2	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, PERICARDIUM		1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, CORONARY ARTERY		0	0	0	0	0	0	0	0	0	1
--NECROSIS, MYOCARDIUM		0	0	1	0	0	0	0	0	0	0
--SEPTIC THROMBUS, AORTIC OUTFLOW		0	0	1	0	0	0	0	0	0	0
--AMYLOIDOSIS		0	0	0	1	0	0	0	0	0	0
--OCCLUSIVE VASCULITIS		0	0	0	0	0	0	1	0	0	0
--SEPTIC VALVULAR ENDOCARDITIS		0	0	0	0	0	0	1	0	0	0
--ATRIAL DILATATION		0	0	0	0	0	0	0	0	1	0

INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE----------					---------FEMALE---------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
SPLEEN (SP)	NUMBER EXAMINED:	55	54	55	55	54	55	55	55	55	55
	NOT REMARKABLE:	2	5	1	5	5	3	1	1	2	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	2	3	5	1	8	14	19	12	11
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	4	2	2	0	0	1	2	2	1
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	1	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--HEMATOPOIESIS		46	42	46	42	44	49	51	50	48	50
--LYMPHOID HYPERPLASIA		0	3	0	2	2	8	3	3	4	3
--LYMPHOID DEPLETION		8	4	6	7	5	5	2	1	3	1
--FIBROSIS, FOCAL		1	0	1	0	0	0	0	0	0	0
--TINGIBLE BODY MACROPHAGES, INCREASED		1	0	0	1	0	0	0	1	0	0
--THROMBUS		0	0	0	0	0	0	0	0	1	0
--AMYLOIDOSIS		0	0	0	1	0	0	0	0	0	0
--ATROPHY		0	0	1	0	0	0	0	0	0	1
--HYPERPLASIA, CAPSULE		1	0	0	0	0	0	0	0	0	0
--MINERALIZED FOCI, CAPSULE		1	0	0	0	0	0	0	0	0	0
--SCAR		0	0	0	0	0	1	0	0	0	0
--OSSEOUS METAPLASIA, FOCAL		0	0	0	0	0	0	0	0	0	1
LIVER (LI)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	18	20	14	23	27	13	12	7	16	11
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	2	4	3	0	7	12	10	8	6
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		4	0	2	1	2	0	0	0	0	0
--B-ADENOMA, HEPATOCELLULAR		5	5	1	5	2	3	2	1	4	4
--M-CARCINOMA, HEPATOCELLULAR		7	9	13	12	11	1	3	3	3	3
--M-CHOLANGIOCARCINOMA		1	0	0	0	0	0	0	0	0	0
--N-LEIOMYOSARCOMA, METASTATIC		0	0	1	0	1	0	0	0	0	0
--INFLAMMATORY FOCI (BACKGROUND)		25	27	18	16	14	36	28	36	23	34
--INCREASED GLYCOGEN		0	0	1	0	0	0	0	1	0	0
--CELLULAR ALTERATION, CLEAR		2	0	0	1	2	1	0	0	2	1

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --									
	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
LIVER (LI)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	18	20	14	23	27	13	12	7	16	11
--CELLULAR ALTERATION, BASOPHILIC		1	5	3	0	1	1	4	3	1	0
--CELLULAR ALTERATION, EOSINOPHILIC		0	0	1	0	0	0	0	0	0	0
--NECROSIS, FOCAL		1	0	1	0	0	0	0	0	2	0
--NECROSIS, CENTRILOBULAR		1	0	0	0	0	0	0	0	1	0
--NECROSIS, PANLOBULAR		0	0	0	0	0	0	0	1	0	0
--NECROSIS, SINGLE-CELL		0	0	1	0	0	0	0	0	0	0
--TUMOR-RELATED NECROSIS		1	4	2	3	3	0	1	0	1	0
--HEMATOPOIESIS		1	1	1	0	0	2	0	1	1	3
--INFLAMMATION, CHRONIC, CAPSULE		0	0	0	0	0	0	0	0	0	1
--HYPERTROPHY, FOCAL		1	0	1	0	0	0	0	0	0	0
--HYPERTROPHY, CENTRILOBULAR		0	0	1	0	0	0	1	0	0	0
--ATROPHY, HEPATOCELLULAR		0	0	0	0	0	0	0	1	0	0
--HYPERTROPHY, PERIPORTAL		0	0	1	0	0	0	0	0	0	1
--LEUKOCYTOSIS		0	0	1	0	0	0	0	0	0	1
--PIGMENTED KUPFFER CELLS		0	0	0	1	0	0	0	1	0	0
--LIPID-LADEN, PIGMENTED MACROPHAGES		0	0	0	0	0	0	2	0	0	0
--HYPERPLASIA, CAPSULE		0	0	0	0	0	0	0	0	0	1
--INCREASED PLOIDY		0	0	0	0	2	0	0	0	0	0
--FOCAL DEGENERATION WITH CHRONIC INFLAMMATION		0	0	0	0	0	0	0	0	0	1
--MICROVESICULATION, HEPATOCELLULAR, CENTRILOBULAR		0	0	1	0	0	0	0	0	0	0
--INCREASED HEPATOCELLULAR MITOTIC ACTIVITY		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, PORTAL BLOOD VESSEL		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE, CENTRILOBULAR		0	0	0	0	0	0	1	0	0	0
--KUPFFER CELL HYPERPLASIA		0	0	0	0	0	0	0	0	1	0
GALLBLADDER (GB)	NUMBER EXAMINED:	41	44	44	38	42	44	40	40	41	38
	NOT REMARKABLE:	41	44	43	38	42	44	38	39	39	38
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	2	1	2	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --									
	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
KIDNEY (KD)	NUMBER EXAMINED:	55	55	54	55	55	55	55	55	55	55
	NOT REMARKABLE:	8	9	10	6	7	36	19	23	27	31
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	1	1	1	6	8	10	3	6
--B-ADENOMA, TUBULAR CELL		0	0	0	0	1	0	0	0	0	0
--M-CARCINOMA, TUBULAR CELL		0	0	0	1	1	0	0	0	0	0
--M-MESENCHYMAL TUMOR		0	0	0	0	0	1	0	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--NEPHROPATHY, CHRONIC PROGRESSIVE		41	41	34	42	41	12	19	21	20	14
--MICROCALCULI, MEDULLA		1	5	2	1	2	3	3	2	3	4
--MINERALIZED FOCI, CORTEX		22	19	19	35	33	0	5	3	1	0
--CYST		1	2	2	8	7	0	1	0	0	0
--HYPERPLASIA, TUBULAR EPITHELIUM		3	1	0	1	6	0	0	0	0	0
--HYPERPLASIA, EPITHELIUM, BOWMAN'S CAPSULE		0	1	0	0	0	0	0	0	0	0
--HYPERPLASIA, PELVIC EPITHELIUM		0	1	0	0	1	0	0	0	0	0
--AMYLOIDOSIS		1	0	1	0	0	1	0	0	1	0
--DILATATION, PELVIS		0	1	1	0	0	0	2	1	0	1
--DILATATION, TUBULES		0	0	1	0	0	0	0	0	0	0
--HYDRONEPHROSIS		0	0	0	0	0	1	1	0	0	0
--ATROPHY, CORTEX		0	0	1	0	0	0	0	0	1	0
--PYELITIS, ACUTE		1	0	0	0	0	0	0	0	0	0
--PYELONEPHRITIS		1	0	1	1	1	0	0	0	0	0
--OSSEOUS METAPLASIA, FOCAL		0	1	0	1	0	0	1	2	1	3
--VACUOLIZATION, TUBULES		1	1	2	0	1	0	0	0	0	0
--PYELITIS, SUBACUTE		0	0	1	0	0	0	0	0	0	0
--TUBULAR PIGMENT		0	0	1	0	0	0	0	0	1	0
--GLOMERULAR PROTEINOSIS		0	0	0	0	0	1	0	0	0	0
--INFARCT		0	0	0	0	0	0	0	0	1	0
--PERIRENAL ABSCESS		0	0	0	0	0	1	0	0	0	0
--PERIRENAL LYMPHOID INFILTRATE		1	0	0	0	0	0	0	0	0	0
--HYALINE DROPLET TUBULAR DEGENERATION		1	0	0	0	0	0	0	0	0	0
*** CONTINUED ON NEXT PAGE ***											

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
KIDNEY (KD)	NUMBER EXAMINED:	55	55	54	55	55	55	55	55	55	55
	NOT REMARKABLE:	8	9	10	6	7	36	19	23	27	31
--CORTICAL SCAR		0	1	1	4	3	0	0	1	1	1
--PYELITIS (WITH PELVIC AND TUBULAR DILATATION)		0	0	0	0	1	0	0	0	0	0
--FOLLICULAR LYMPHOID AGGREGATE, SUBCAPSULAR		0	0	0	0	0	1	0	0	0	0
--FIBROSIS, FOCAL, MEDULLA		0	1	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, CAPSULE		0	0	0	0	0	0	1	0	0	0
--PROTEIN RESORPTION DROPLETS		0	0	0	0	0	0	0	1	0	0
STOMACH, NONGL (SU)	NUMBER EXAMINED:	54	52	53	54	54	54	55	55	55	55
	NOT REMARKABLE:	44	45	36	34	29	49	41	23	26	16
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	1	0	0	1	0	2	4
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	0	0	0	0	0	1	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--HYPERPLASIA, MARGINAL PLATE		9	4	8	14	16	3	8	14	17	12
--HYPERPLASIA, EPITHELIAL		1	2	8	5	12	1	4	18	12	26
--HYPERKERATOSIS		0	2	0	1	4	0	2	2	1	11
--MICROABSCESSATION, MARGINAL PLATE		1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, SUBMUCOSAL		0	0	0	1	1	0	0	0	0	0
--EDEMA		0	0	0	0	0	1	0	0	0	0
--EDEMA, MARGINAL PLATE		0	0	0	0	0	0	0	0	0	1
--KERATIN CYST		0	0	1	1	0	0	0	2	0	4
--ABERRANT DUCT, MARGINAL PLATE		0	1	0	0	0	0	0	0	0	0
STOMACH, GL (ST)	NUMBER EXAMINED:	55	55	55	55	52	54	55	55	54	54
	NOT REMARKABLE:	47	47	36	39	28	41	31	30	33	36
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	1	0	0	1	1	1	3
--M-LEIOMYOSARCOMA		0	0	0	0	1	0	0	0	0	0
--HYPERPLASIA, MUCOSAL EPITHELIUM		2	2	13	9	19	3	15	20	17	13
--ADENOMATOUS HYPERPLASIA, FOCAL		0	0	0	1	4	0	0	0	0	1
*** CONTINUED ON NEXT PAGE ***											

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
STOMACH, GL (ST)	NUMBER EXAMINED:	55	55	55	55	52	54	55	55	54	54
	NOT REMARKABLE:	47	47	36	39	28	41	31	30	33	36
--HYPERPLASIA, MARGINAL PLATE		2	3	2	1	0	0	2	5	2	0
--DILATED GLAND(S)		0	2	5	4	4	5	5	5	5	4
--EROSION		2	2	1	2	1	3	4	1	2	1
--ULCER		1	0	1	0	1	2	2	0	1	0
--INFLAMMATION, ACUTE, SUBMUCOSA		1	0	0	0	0	0	0	0	0	0
--EDEMA		0	0	0	0	0	1	0	0	0	0
--ARTERITIS, SEROSA		0	0	0	0	0	0	0	1	0	0
--SQUAMOUS METAPLASIA		0	0	0	0	0	0	1	0	0	1
DUODENUM (DU)	NUMBER EXAMINED:	47	52	49	50	49	52	50	54	49	48
	NOT REMARKABLE:	47	52	48	49	48	52	49	54	48	47
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	0	0	1	1
--B-POLYP		0	0	0	0	0	0	1	0	0	0
--M-CARCINOMA		0	0	0	1	0	0	0	0	0	0
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
JEJUNUM (JE)	NUMBER EXAMINED:	47	52	49	48	48	49	51	54	49	48
	NOT REMARKABLE:	47	52	49	47	48	49	51	51	48	48
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	3	1	0
ILEUM (IL)	NUMBER EXAMINED:	48	52	49	46	48	49	50	52	47	48
	NOT REMARKABLE:	48	52	49	46	48	49	50	51	47	47
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	1
--NECROSIS, TRANSMURAL		0	0	0	0	0	0	0	0	0	1

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
PANCREAS (PA)	NUMBER EXAMINED:	55	54	55	55	53	55	55	55	55	55
	NOT REMARKABLE:	54	51	52	53	52	49	48	50	49	45
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	3	1	0	3	4	4	3	4
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	0	1	0	0	0	0	0	0
--B-ADENOMA, ISLET CELL		0	0	0	0	0	1	0	0	0	0
--N-FIBROSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--I-SARCOMA, INVASIVE		0	0	0	0	0	0	0	0	0	1
--CYTOPLASMIC ALTERATION, BASOPHILIC		1	0	0	0	0	0	1	0	0	0
--ACINAR ATROPHY		0	1	0	0	0	1	0	0	1	2
--AMYLOIDOSIS		0	0	0	0	1	0	0	0	0	0
--HYPERPLASIA, ISLET CELLS		0	0	0	0	0	0	1	0	0	0
--HYPERTROPHY, FOCAL, ACINAR CELLS		1	0	0	0	0	0	0	0	1	0
--VACUOLES, ACINAR CELLS		0	0	0	0	0	2	0	1	0	0
--CYST(S)		0	0	0	0	0	0	1	0	0	1
--ARTERITIS, CHRONIC		0	1	0	0	0	0	0	0	1	0
CECUM (CE)	NUMBER EXAMINED:	53	54	50	48	51	51	51	54	52	54
	NOT REMARKABLE:	53	54	50	47	50	51	51	53	49	54
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	2	0
--LYMPHOID HYPERPLASIA		0	0	0	0	0	0	0	0	1	0
--EPITHELIAL HYPERPLASIA		0	0	0	1	1	0	0	0	0	0
COLON (CO)	NUMBER EXAMINED:	53	53	52	51	54	55	55	55	54	54
	NOT REMARKABLE:	53	53	51	51	54	55	54	55	54	54
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	0	0	0	0
--CHRONIC PROLIFERATIVE PERITONITIS		0	0	0	0	0	0	1	0	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
RECTUM (RE)	NUMBER EXAMINED:	54	53	52	49	55	54	55	54	55	55
	NOT REMARKABLE:	54	53	52	49	55	54	55	54	55	55
LN, MESENTERIC (MS)	NUMBER EXAMINED:	51	54	53	51	49	55	52	49	49	54
	NOT REMARKABLE:	24	20	25	23	15	45	40	33	36	33
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	2	5	4	0	7	9	11	6	12
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
--N-RENAL TUBULAR CARCINOMA, METASTATIC		0	0	0	1	0	0	0	0	0	0
--MESENTERIC DISEASE		26	31	23	23	30	3	2	5	7	9
--HEMORRHAGE		0	0	1	1	2	0	0	0	0	0
--NECROSIS, LYMPHOID		1	0	0	0	0	0	0	0	0	0
--PIGMENTED MACROPHAGES		0	0	0	0	0	0	1	0	0	0
--HYPERPLASIA, LYMPHOID		1	2	0	1	2	0	0	0	0	0
--PLASMACYTOSIS		0	1	0	0	0	0	0	0	0	0
--SINUS ECTASIA		0	0	0	1	0	0	0	0	0	0
--LEUKEMOID REACTION		0	0	0	0	0	0	0	0	0	1
SALIV GL, MANDIB (SG)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	54	55
	NOT REMARKABLE:	54	55	54	55	54	54	53	53	53	54
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	0	0	1	2	1	1	1
--INFLAMMATION, CHRONIC, BLOOD VESSEL		0	0	0	0	1	0	0	1	0	0
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	49	48	43	38	45	50	47	50	46	51
	NOT REMARKABLE:	47	46	38	35	45	43	41	36	40	45
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	2	4	1	0	7	6	12	6	5
--N-RENAL TUBULAR CARCINOMA, METASTATIC		0	0	0	1	0	0	0	0	0	0
--HYPERPLASIA, LYMPHOID		0	0	1	1	0	0	0	1	0	1
--HYPERPLASIA, LYMPHOPLASMACYTIC		0	0	0	0	0	0	0	1	0	0

INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
THYMUS (TH)	NUMBER EXAMINED:	39	41	35	34	41	49	46	44	44	43
	NOT REMARKABLE:	33	34	29	32	31	38	37	31	34	37
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	1	1	3	6	12	5	5
--N-CHOLANGIOCARCINOMA, METASTATIC		1	0	0	0	0	0	0	0	0	0
--N-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--LYMPHOID DEPLETION		1	2	4	1	2	6	3	1	4	1
--NECROSIS, LYMPHOID		0	0	2	0	0	0	0	0	1	0
--CYST(S)		4	5	0	0	7	0	0	0	0	0
--MEDULLARY HYPERPLASIA		0	0	0	0	0	2	0	0	0	0
AORTA, THORACIC (AO)	NUMBER EXAMINED:	53	53	53	51	54	55	53	52	55	54
	NOT REMARKABLE:	53	53	53	50	54	55	53	51	55	54
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	1	0	0
EYE (EY)	NUMBER EXAMINED:	52	51	51	53	53	55	55	53	51	52
	NOT REMARKABLE:	51	51	50	53	52	54	55	52	48	50
--ULCER, CORNEA		0	0	0	0	0	0	0	0	1	0
--HEMORRHAGE AND SUBACUTE INFLAMMATION, PERIORBITAL		0	0	1	0	0	0	0	0	0	0
--LENTICULAR DEGENERATION		0	0	0	0	1	1	0	0	1	1
--CORNEAL VASCULARIZATION		1	0	0	0	0	0	0	0	1	0
--CHORISTOMA (BONE/MARROW)		0	0	0	0	0	0	0	0	0	1
--INFLAMMATION, SUBACUTE, RETROBULBAR		0	0	0	0	0	0	0	1	0	0
HARDERIAN GLAND (HG)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	54	55
	NOT REMARKABLE:	53	48	52	50	48	53	48	45	49	46
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	4	3	1	1
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	0	0	0	0	0	1	0	0
--B-ADENOMA		1	3	3	4	4	2	2	2	1	5
--M-CARCINOMA		1	2	0	1	1	0	0	1	2	2

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INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
HARDERIAN GLAND (HG)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	54	55
	NOT REMARKABLE:	53	48	52	50	48	53	48	45	49	46
--HYPERPLASIA, FOCAL		0	2	0	0	2	0	1	2	0	1
--INFLAMMATION, CHRONIC		0	1	0	0	0	0	0	1	0	0
--INCREASED PIGMENT		0	0	0	0	0	0	0	0	1	0
LACRIMAL GL, EX (EO)	NUMBER EXAMINED:	53	53	55	49	49	54	46	51	48	50
	NOT REMARKABLE:	53	52	55	47	49	53	45	48	42	45
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	1	2	4	4
--NECROSIS, GLANDULAR CELLS		0	1	0	1	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	0	1	0	0	1	1
--INFLAMMATION, SUBACUTE		0	1	0	0	0	0	0	0	1	0
--CYST		0	0	0	0	0	0	0	1	0	0
MUSCLE, SKELETAL (SM)	NUMBER EXAMINED:	55	54	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	49	49	51	53	50	48	46	47	51	46
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	3	1	0	0
--INFLAMMATION, CHRONIC		3	5	3	0	3	3	6	4	4	3
--ATROPHY		0	0	0	0	0	2	0	1	0	3
--DEGENERATION, MUSCLE FIBER(S)		3	1	0	1	1	2	0	1	1	0
--FOCAL MINERALIZATION		0	0	0	1	1	0	0	1	0	1
--FIBROSIS		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, ACUTE AND CHRONIC		0	0	0	0	0	0	0	0	0	1
--MYXOMATOUS INFILTRATION		0	0	0	0	0	0	0	0	0	1
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	55	53	55	54	55	55	55	52	55	52
	NOT REMARKABLE:	16	15	15	15	10	14	9	6	12	4
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	1	1	2	0	3
--AXON DEGENERATION		39	38	39	38	45	41	45	46	43	47

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INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	55	53	55	54	55	55	55	52	55	52
	NOT REMARKABLE:	16	15	15	15	10	14	9	6	12	4
--LYMPHOCYTIC INFILTRATE		0	0	2	0	0	3	1	0	0	4
TONGUE (TO)	NUMBER EXAMINED:	55	54	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	55	54	55	55	55	55	54	54	55	55
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	0	0
--ARTERITIS		0	0	0	0	0	0	0	1	0	0
TESTIS (TE)	NUMBER EXAMINED:	55	55	55	55	55	0	0	0	0	0
	NOT REMARKABLE:	41	54	45	49	39	0	0	0	0	0
--B-BENIGN INTERSTITIAL CELL TUMOR		0	0	0	1	1	0	0	0	0	0
--DEGENERATION, SEMINIFEROUS TUBULES		14	1	10	5	14	0	0	0	0	0
--ATROPHY, UNILATERAL		1	0	0	0	1	0	0	0	0	0
EPIDIDYMIS (EP)	NUMBER EXAMINED:	55	55	54	54	54	0	0	0	0	0
	NOT REMARKABLE:	52	53	52	54	51	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	0	0	0	0
--M-SARCOMA		0	0	0	0	1	0	0	0	0	0
--HYPOSPERMIA		3	0	0	0	0	0	0	0	0	0
--XANTHOGRANULOMA		0	0	0	0	1	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	1	1	0	2	0	0	0	0	0
--X-VASXULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	1	0	0	0	0	0	0	0	0
PROSTATE (PR)	NUMBER EXAMINED:	54	53	54	54	54	0	0	0	0	0
	NOT REMARKABLE:	50	52	50	47	51	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	0	0	0	0

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INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
PROSTATE (PR)	NUMBER EXAMINED:	54	53	54	54	54	0	0	0	0	0
	NOT REMARKABLE:	50	52	50	47	51	0	0	0	0	0
--INFLAMMATION, ACUTE		0	0	1	0	1	0	0	0	0	0
--INFLAMMATION, CHRONIC		4	1	1	7	2	0	0	0	0	0
--INFLAMMATION, CHRONIC, PERIURETHRAL		0	0	1	0	0	0	0	0	0	0
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	54	55	55	55	55	0	0	0	0	0
	NOT REMARKABLE:	51	55	52	53	55	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	0	0	0	0	0
--M-LEIOMYOSARCOMA		0	0	1	0	0	0	0	0	0	0
--DILATATION		3	0	1	2	0	0	0	0	0	0
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	0	52	54	54	55	52
	NOT REMARKABLE:	0	0	0	0	0	9	8	1	6	3
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	4	3	2	1
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	0	0	0	1	0	1	0	3
--B-CYSTADENOMA		0	0	0	0	0	1	2	2	1	0
--SENILE ATROPHY		0	0	0	0	0	37	39	46	39	44
--CYST(S)		0	0	0	0	0	12	14	17	17	17
--CYST, BURSA		0	0	0	0	0	1	0	3	0	0
--HEMATOCYST(S)		0	0	0	0	0	9	15	11	16	14
--HYPERPLASIA, STROMA		0	0	0	0	0	0	0	0	1	0
--INFLAMMATION, SUBACUTE/CHRONIC, BURSA		0	0	0	0	0	2	0	0	0	0
--ABSCESS		0	0	0	0	0	1	0	0	0	1
--HYPERPLASIA, INTERSTITIAL CELL		0	0	0	0	0	1	0	0	0	0
--AMYLOID		0	0	0	0	0	0	0	0	0	1
--ADENOMATOUS HYPERPLASIA, OVIDUCT		0	0	0	0	0	0	0	1	0	0

INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	0	55	55	55	55	55
	NOT REMARKABLE:	0	0	0	0	0	4	2	1	7	3
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	3	0	2	1
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	0	0	0	0	0	0	1	0
--B-ENDOMETRIAL STROMAL POLYP		0	0	0	0	0	1	0	2	2	2
--B-ADENOMATOID POLYP		0	0	0	0	0	0	0	1	0	0
--B-LEIOMYOMA		0	0	0	0	0	0	0	1	0	0
--M-CARCINOMA		0	0	0	0	0	2	0	0	1	0
--M-ENDOMETRIAL STROMAL SARCOMA		0	0	0	0	0	0	0	0	0	1
--M-OSTEOSARCOMA		0	0	0	0	0	0	0	0	0	1
--HYPERPLASIA, CYSTIC ENDOMETRIAL		0	0	0	0	0	50	49	52	45	48
--DILATATION		0	0	0	0	0	4	10	10	5	7
--ABSCESSED GLANDS		0	0	0	0	0	1	0	0	1	0
--HEMATOCYST		0	0	0	0	0	0	0	0	0	1
--SUPPURATIVE EXUDATE		0	0	0	0	0	0	1	0	0	0
--FIBROSIS, ENDOMETRIAL STROMA		0	0	0	0	0	0	0	1	0	0
--HYPERPLASIA, ENDOMETRIAL STROMA		0	0	0	0	0	0	0	1	0	0
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	0	54	53	51	50	55
	NOT REMARKABLE:	0	0	0	0	0	53	52	49	50	52
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	2	0	2
--POLYPOID SQUAMOUS HYPERPLASIA		0	0	0	0	0	1	0	0	0	0
--MYXOMATOUS DEGENERATION		0	0	0	0	0	0	0	0	0	1
URINARY BLADDER (UB)	NUMBER EXAMINED:	54	53	55	54	55	52	54	52	51	52
	NOT REMARKABLE:	52	53	54	51	53	50	51	44	49	46
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0	2	3	5	2	5
--INFLAMMATION, ACUTE		1	0	0	0	1	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	1	0	0	0	3	0	0
--INFLAMMATION, ACUTE AND CHRONIC		1	0	0	1	1	0	0	0	0	0

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INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE---------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
URINARY BLADDER (UB)	NUMBER EXAMINED:	54	53	55	54	55	52	54	52	51	52
	NOT REMARKABLE:	52	53	54	51	53	50	51	44	49	46
--UROTHELIAL HYPERPLASIA (RELATED TO INFLAMMATION)		0	0	0	0	1	0	0	0	0	0
--EXTREME DISTENSION		0	0	0	1	0	0	0	0	0	1
MAMMARY, FEMALE (MF)	NUMBER EXAMINED:	0	0	0	0	0	47	44	28	44	48
	NOT REMARKABLE:	0	0	0	0	0	37	34	18	34	32
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	2	1	0	4
--B-ADENOMA		0	0	0	0	0	1	0	0	0	0
--B-FIBROADENOMA		0	0	0	0	0	0	1	1	0	0
--M-CARCINOMA		0	0	0	0	0	0	0	0	0	1
--LACTATION		0	0	0	0	0	4	7	6	10	5
--HYPERPLASTIC ALVEOLAR NODULES		0	0	0	0	0	3	0	2	1	3
--FIBROCYSTIC-LIKE CHANGES		0	0	0	0	0	3	2	0	1	3
SKIN (SK)	NUMBER EXAMINED:	55	53	55	54	55	55	55	55	55	55
	NOT REMARKABLE:	53	53	52	53	52	53	54	55	53	50
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	1	1
--M-BASAL CELL CARCINOMA		0	0	0	0	0	1	0	0	0	0
--M-LEIOMYOSARCOMA, METASTATIC		0	0	0	0	1	0	0	0	0	0
--EDEMA		2	0	0	0	1	0	0	0	2	0
--ULCERATION		0	0	1	1	0	0	0	0	0	0
--SUPERFICIAL PYODERMA		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC, SUBCUTANEOUS		0	0	1	0	0	0	0	0	0	2
--INFLAMMATION, CHRONIC, ADNEXA		0	0	0	0	1	0	0	0	0	0
--DILATED ADNEXAL GLANDS		0	0	0	0	0	1	0	0	0	0
--DEGENERATION, SUBCUTANEOUS MUSCLE		0	0	0	0	0	0	0	0	0	1
--MYXOMATOUS INFILTRATION, SUBCUTANEOUS		0	0	0	0	0	0	0	0	0	1
--INFLAMMATION, GRANULOMATOUS, SUBCUTANEOUS		0	0	1	0	0	0	0	0	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		SEX:	MALE					FEMALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
		NUMBER:	55	55	55	55	55	55	55	55	55	55
BONE, STERNUM (SB)	NUMBER EXAMINED:		55	54	55	55	55	55	55	55	54	54
	NOT REMARKABLE:		55	54	55	55	55	2	4	2	6	4
--X-HEMATOPOIETIC NEOPLASIA			0	0	0	0	0	0	1	1	0	0
--FIBRO-OSSEOUS CHANGE (BACKGROUND)			0	0	0	0	0	53	50	52	48	50
MARROW, STERNUM (SE)	NUMBER EXAMINED:		55	54	55	55	55	55	55	55	54	54
	NOT REMARKABLE:		51	53	53	51	52	50	53	52	49	51
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	0	0	0	1	2	2	0	2
--HYPERPLASIA			4	1	2	2	2	2	1	0	3	0
--HYPERPLASIA, MYELOCYTIC			0	0	0	2	1	2	0	1	2	1
BONE, FEMUR (FE)	NUMBER EXAMINED:		55	54	55	55	55	55	55	55	55	55
	NOT REMARKABLE:		52	51	53	55	51	17	17	14	18	16
--X-HEMATOPOIETIC NEOPLASIA			0	0	0	0	0	0	1	0	0	0
--X-VASCULAR NEOPLASM (SEE VASCULAR NEOPLASIA FOR TYPE)			0	1	0	0	0	0	0	0	0	0
--M-OSTEOSARCOMA			1	0	0	0	0	0	1	0	0	1
--FIBRO-OSSEOUS CHANGE (BACKGROUND)			2	2	1	0	4	38	35	41	36	36
--FRACTURE WITH CALLUS			0	0	0	0	0	0	2	0	1	0
--INFLAMMATION, ACUTE, JOINT CAPSULE			0	0	0	0	0	0	0	0	0	1
--INFLAMMATION, CHRONIC, JOINT CAPSULE			0	0	0	0	0	1	0	0	0	0
--HYPEROSTOSIS			0	0	0	0	0	0	0	0	0	1
--ENDOSTEAL HYPEROSTOSIS			0	0	1	0	0	0	0	0	0	0
--THROMBUS			0	0	0	0	0	0	1	0	0	0
--HYPEROSTOTIC EXOSTOSIS			0	0	0	0	0	0	0	1	0	0
MARROW, FEMUR (FM)	NUMBER EXAMINED:		55	53	55	55	55	55	55	55	55	55
	NOT REMARKABLE:		50	50	51	52	51	50	50	50	49	48
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	0	0	0	0	1	3	3	0	3
--X-VASCULAR NEOPLASM (SEE MULTIPLE ORGANS)			0	2	2	0	0	0	1	0	0	0

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		SEX:	MALE					FEMALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
		NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***												
MARROW, FEMUR (FM)	NUMBER EXAMINED:		55	53	55	55	55	55	55	55	55	55
	NOT REMARKABLE:		50	50	51	52	51	50	50	50	49	48
--HYPERPLASIA			3	1	1	1	3	2	1	1	4	2
--HYPERPLASIA, MYELOCYTIC			1	0	1	2	1	2	0	1	1	2
--FOCAL RAREFACTION			0	0	0	0	0	0	1	0	1	0
NASAL TURBINATE (NT)	NUMBER EXAMINED:		55	53	55	55	55	55	55	55	55	55
	NOT REMARKABLE:		52	50	52	49	54	54	51	52	53	50
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	1	0	0	0	1	1	0	1
--B-ODONTOMA			0	1	0	0	0	0	0	0	0	0
--M-OSTEOSARCOMA			0	1	0	0	0	0	1	0	0	0
--M-SARCOMA, CRANIAL MARROW CAVITY			0	0	0	1	0	0	0	0	0	0
--I-CARCINOMA, INVASIVE HARDERIAN GLAND			0	0	0	0	0	0	0	0	0	1
--I-OSTEOSARCOMA, INVASIVE			0	0	0	0	0	0	0	0	0	1
--FOREIGN MATERIAL AND EXUDATE			0	1	1	0	0	0	0	0	0	0
--EXUDATE			1	0	0	0	0	1	1	1	0	0
--ULCERATION			1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, ACUTE			1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, ACUTE, NASOLACRIMAL DUCT			0	0	0	1	0	0	1	0	0	0
--DENTAL DYSPLASIA			0	1	0	1	0	0	0	0	1	1
--ALVEOLAR ABSCESS			1	0	1	4	1	0	0	0	1	0
--PERIOSTEAL HYPEROSTOSIS, CRANIUM			0	0	0	0	0	0	0	0	0	1
--FIBROSIS, GLANDS			0	1	0	0	0	0	0	0	0	0
--EPITHELIAL HYPERPLASIA, NASOLACRIMAL DUCT			0	0	0	0	0	0	0	1	0	0
HEMATO NEOPLASIA (HN)	NUMBER EXAMINED:		55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:		51	51	49	48	52	43	39	33	43	43
--M-LYMPHOMA			3	3	4	6	3	8	14	19	9	11
--M-LEUKEMIA, GRANULOCYTIC			0	0	0	0	0	1	0	1	0	0
--M-SARCOMA, HISTIOCYTIC			1	1	1	0	0	1	0	1	1	0

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INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
HEMATO NEOPLASIA (HN)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	51	51	49	48	52	43	39	33	43	43
--M-MALIGNANT FIBROUS HISTIOCYTOMA		0	0	0	1	0	0	0	1	0	0
--M-LYMPHOMA, HISTIOCYTIC		0	0	0	0	0	1	1	0	1	0
--M-LEUKEMIA		0	0	0	0	0	1	1	0	1	1
--M-PLASMACYTOMA		0	0	1	0	0	0	0	0	0	0
^DEATH COMMENT (DC)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--SCHEDULED SACRIFICE		38	42	35	32	42	39	36	43	33	33
--UNDETERMINED		7	8	9	11	6	9	11	5	10	10
--HEMATOPOIETIC NEOPLASIA		1	2	2	2	1	2	5	6	5	3
--AMYLOIDOSIS		1	0	1	0	0	0	0	0	1	0
--OVARIAN ABSCESS		0	0	0	0	0	1	0	0	0	1
--SCHWANNOMA, MALIGNANT		0	0	0	0	0	1	0	0	0	0
--HEMANGIOSARCOMA		2	1	2	3	2	0	0	0	1	0
--CHOLANGIOCARCINOMA		1	0	0	0	0	0	0	0	0	0
--LIVER NECROSIS		1	0	0	0	0	0	0	1	0	0
--PYELONEPHRITIS		1	0	0	1	1	0	0	0	0	0
--FIBROSARCOMA		0	0	0	0	0	1	0	0	1	2
--FIBROSARCOMA, BIOMEDIC IMPLANT		0	0	0	0	0	0	0	0	0	1
--OSTEOSARCOMA		1	0	0	0	0	0	1	0	1	2
--HEPATOCELLULAR CARCINOMA		2	3	4	7	4	0	0	1	1	1
--RHABDOMYOSARCOMA		0	0	0	1	0	0	0	0	0	0
--MEDIASTINAL ABSCESS (GAVAGE RELATED)		0	0	0	0	0	1	0	0	0	0
--PHEOCHROMOCYTOMA		0	0	0	0	0	0	1	0	0	0
--HISTIOCYTIC PNEUMONIA		0	0	0	0	0	0	0	0	1	0
--BRONCHIOLOALVEOLAR CARCINOMA		0	0	0	0	0	0	0	0	1	0
--SEPTIC THROMBUS, HEART		0	0	1	0	0	0	0	0	0	0
--HYDRONEPHROSIS		0	0	0	0	0	1	0	0	0	0
--UTERINE CARCINOMA		0	0	0	0	0	1	0	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
^DEATH COMMENT (DC)	NUMBER EXAMINED:	55	55	55	55	55	55	55	55	55	55
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--SKIN ULCER, ABSCESS, PERINEAL REGION		0	0	0	0	0	0	1	0	0	0
--HARDERIAN GLAND CARCINOMA		0	0	0	0	0	0	0	0	0	1
--PITUITARY NEOPLASIA		0	0	0	0	0	0	0	0	1	0
--PERITONITIS		0	0	0	0	0	0	0	0	1	0
--URINARY TRACT OBSTRUCTION		0	0	0	0	0	0	0	0	0	1
--LEIOMYOSARCOMA		0	0	1	0	0	0	0	0	0	0
--ABSCESS, PREPUTIAL GLAND		0	1	0	0	0	0	0	0	0	0
--RENAL TUBULAR CELL CARCINOMA		0	0	0	1	0	0	0	0	0	0
--SEPTIC VALVULAR ENDOCARDITIS		0	0	0	0	0	0	1	0	0	0
--PULMONARY HEMORRHAGE		0	0	0	0	0	0	1	0	0	0
VASC NEOPLASIA (VN)	NUMBER EXAMINED:	5	4	4	4	3	2	1	4	4	4
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--N-HEMANGIOMA		1	0	0	0	0	1	0	0	1	1
--M-HEMANGIOSARCOMA		4	3	4	4	3	1	1	3	3	3
--B-HEMANGIOMA		0	1	0	0	0	0	0	1	0	0
SKIN, OTHER (SS)	NUMBER EXAMINED:	20	8	13	15	15	27	17	26	21	19
	NOT REMARKABLE:	18	8	12	11	14	25	12	24	20	18
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0	0	0
--M-SQUAMOUS CELL CARCINOMA		0	0	0	0	0	0	0	1	0	0
--ULCERATION, INFLAMMATION		0	0	0	2	0	0	2	0	0	0
--EDEMA, FOOT		0	0	0	0	0	0	0	0	1	0
--EDEMA		2	0	0	0	0	2	0	0	0	1
--ULCERATION, TAIL		0	0	1	0	0	0	1	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	0	0	0	1	0	0	0	0	0
--INFLAMMATION, SUBACUTE, SUBCUTANEOUS		0	0	0	1	0	0	0	0	0	0
--ACANTHOSIS		0	0	0	1	0	0	0	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE----------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
*** FROM PREVIOUS PAGE ***											
SKIN, OTHER (SS)	NUMBER EXAMINED:	20	8	13	15	15	27	17	26	21	19
	NOT REMARKABLE:	18	8	12	11	14	25	12	24	20	18
--KERATIN CYST		0	0	0	1	0	0	0	0	0	0
--ESCHAR, TAIL		0	0	0	0	0	0	1	0	0	0
--ULCERATION, EAR		0	0	0	0	0	0	0	1	0	0
LN, OTHER (LNO)	NUMBER EXAMINED:	3	2	5	4	1	6	6	6	6	4
	NOT REMARKABLE:	0	0	0	1	0	0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	2	4	1	1	5	5	6	5	3
--X-VASCULAR NEOPLOASM (SEE VASCULAR NEOPLASIA FOR TYPE)		0	0	0	0	0	1	0	0	0	0
--N-FIBROSARCOMA, BIOMEDIC IMPLANT, METASTATIC		1	0	0	0	0	0	0	0	0	0
--SINUS ECTASIA		0	0	1	0	0	0	0	0	0	1
--HYPERPLASIA, LYMPHOID		0	0	0	2	0	0	0	0	0	0
--HYPERPLASIA, LYMPHOPLASMACYTIC		0	0	0	0	0	0	0	0	1	0
SUBCUTANEOUS TIS (SQ)	NUMBER EXAMINED:	4	1	0	4	1	4	1	1	4	3
	NOT REMARKABLE:	1	0	0	1	1	0	0	0	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	0	1	0
--B-FIBROMA		0	0	0	1	0	0	0	0	0	0
--M-SCHWANNOMA, MALIGNANT		0	0	0	0	0	1	0	0	0	0
--M-FIBROSARCOMA		0	0	0	0	0	1	0	1	1	1
--M-FIBROSARCOMA, BIOMEDIC IMPLANT		1	0	0	0	0	0	1	0	0	1
--M-RHABDOMYOSARCOMA		0	0	0	1	0	0	0	0	0	0
--M-OSTEOSARCOMA		0	0	0	0	0	0	0	0	1	0
--ABSCESS		0	1	0	0	0	1	0	0	0	0
--EDEMA		2	0	0	0	0	1	0	0	0	0

INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-107
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
SEX:		----------MALE----------					---------FEMALE--------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		55	55	55	55	55	55	55	55	55	55
CAVITY, ABDOM (PC)	NUMBER EXAMINED:	0	0	1	1	0	2	2	1	6	6
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	1	0	2	1	1	4	3
--M-FIBROSARCOMA		0	0	0	0	0	0	0	0	0	1
--STRATITIS, MESENTERIC		0	0	0	0	0	0	0	0	1	1
--PERITONITIS, ACUTE AND CHRONIC		0	0	0	0	0	0	0	0	1	0
--HEMORRHAGE		0	0	0	0	0	0	1	0	0	0
PENIS (PE)	NUMBER EXAMINED:	5	0	2	1	1	0	0	0	0	0
	NOT REMARKABLE:	2	0	2	0	1	0	0	0	0	0
--ULCER/INFLAMMATION		3	0	0	0	0	0	0	0	0	0
--ULCERATION, SUPPURATIVE INFLAMMATION, PREPUCE		0	0	0	1	0	0	0	0	0	0
CERVIX (CV)	NUMBER EXAMINED:	0	0	0	0	0	1	0	3	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	2	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
--M-LEIOMYOSARCOMA		0	0	0	0	0	0	0	1	0	0
--FIBROSIS		0	0	0	0	0	1	0	0	0	0
DIAPHRAGM (DP)	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	0	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--N-OSTEOSARCOMA, METASTATIC		0	0	0	0	0	0	0	0	0	1
PREPUTIAL GLAND (PG)	NUMBER EXAMINED:	2	1	3	2	3	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	1	0	0	0	0	0	0
--ABSCESS		2	1	2	1	2	0	0	0	0	0
--DILATATION		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	1	0	0	0	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

		-- NUMBER-OF-ANIMALS-AFFECTED --									
TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-107 DEATH=ALL;FIND=ALL;SUBSET=ALL	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	55	55	55	55	55	55	55	55	55	55
MEDIASTINUM (MT)	NUMBER EXAMINED:	0	0	0	0	0	1	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--ABSCESS (GAVAGE RELATED)		0	0	0	0	0	1	0	0	0	0
URETER (UE)	NUMBER EXAMINED:	0	0	2	1	0	1	0	1	0	0
	NOT REMARKABLE:	0	0	1	0	0	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	1	0	0
--INFLAMMATION, CHRONIC		0	0	1	0	0	0	0	0	0	0
--INFLAMMATION, ACUTE		0	0	0	1	0	0	0	0	0	0
NERVE, OPTIC (ON)	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	1	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--PERINEURAL LYMPHOCYTIC INFILTRATE		0	0	0	0	0	0	0	0	1	1
CRANIUM (CR)	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	0	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--M-OSTEOSARCOMA		0	0	0	0	0	0	0	0	0	1

*** END OF LIST ***

APPEARS THIS WAY
ON ORIGINAL

APPENDIX 2: Microscopic Observations in Male and Female Rats.

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED -							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** TOP OF LIST ***									
BRAIN (BR)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	53	47	49	52	30	32	38	46
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	2	0	0	0	0
--M-OLIGODENDROGLIOMA		0	1	0	0	0	0	0	0
--M-ASTROCYTOMA		1	1	1	2	1	0	0	2
--M-GRANULAR CELL TUMOR		0	1	1	0	0	1	0	0
--COMPRESSION, VENTRAL		9	14	14	9	34	31	27	18
--ABSCESS		0	0	0	0	0	1	0	0
--HEMORRHAGE		0	0	0	0	0	1	0	1
--MINERALIZATION		0	0	0	0	0	1	0	0
--INFLAMMATION, CHRONIC		0	1	0	0	0	0	0	0
--CYST		1	0	0	0	0	0	0	0
CORD, CERVICAL (CS)	NUMBER EXAMINED:	63	65	65	65	65	65	64	65
	NOT REMARKABLE:	62	64	65	65	65	64	64	65
--HEMORRHAGE		0	1	0	0	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0	1	0	0
--MINERALIZATION		1	0	0	0	0	0	0	0
CORD, THORACIC (TC)	NUMBER EXAMINED:	63	65	65	65	65	65	64	65
	NOT REMARKABLE:	61	65	65	65	65	64	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0	1	0	0
--MEDIA, MINERALIZATION, VESSEL		1	0	0	0	0	0	0	0
--GLIOSIS		0	0	0	0	0	0	0	1
CORD, LUMBAR (LC)	NUMBER EXAMINED:	63	65	65	65	65	65	64	65
	NOT REMARKABLE:	63	65	65	65	65	64	64	64
--I-ASTROCYTOMA		0	0	0	0	0	0	0	1

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
CORD, LUMBAR (LC)	NUMBER EXAMINED:	63	65	65	65	65	65	64	65
	NOT REMARKABLE:	63	65	65	65	65	64	64	64
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0	1	0	0
PITUITARY (PI)	NUMBER EXAMINED:	64	65	65	64	64	65	64	64
	NOT REMARKABLE:	24	22	28	36	7	13	12	20
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	0	0	0
--B-ADENOMA		26	28	29	22	52	47	47	37
--HYPERPLASIA		9	15	8	5	5	5	4	5
--CYST		6	2	1	2	0	2	0	2
--ANGIECTASIS		0	0	0	0	0	0	1	0
--I-ASTROCYTOMA		0	0	0	0	0	0	0	1
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	1	0	2	0	0	0	0	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	1	1	3	0	0	0	1
--B-ADENOMA		0	0	0	0	3	0	0	0
--N-CARCINOSARCOMA, MAMMARY		0	0	0	0	0	1	0	0
--LIPOGENIC PIGMENTATION		64	65	62	63	65	65	63	62
--VACUOLIZATION		27	40	32	19	27	23	26	16
--CONGESTION/ANGIECTASIS		48	54	50	57	65	65	63	61
--THROMBOSIS		7	2	1	0	41	48	50	42
--MINERALIZATION		1	0	0	1	0	2	1	0
--HEMATOPOIESIS, EXTRAMEDULLARY		2	0	2	2	4	0	3	1
--BONE SPICULE		1	0	0	0	0	0	0	0
--HYPERPLASIA		0	3	1	0	0	1	2	0
--HYPERTROPHY, FOCAL		5	5	1	3	3	1	5	1
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	1	0	0
--NECROSIS		0	1	0	0	0	1	0	2

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	65	65	64	65	65	65	65	65
	NOT REMARKABLE:	51	41	50	58	63	58	57	63
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	0	0	0
--B-PHEOCHROMOCYTOMA		9	14	7	6	1	1	5	0
--M-MALIGNANT PHEOCHROMOCYTOMA		2	3	2	2	0	0	0	1
--HYPERPLASIA		4	9	5	2	1	6	4	1
--UNILATERALLY EXAMINED		0	1	1	0	1	0	1	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	1	0	0
THYROID (TY)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	23	26	27	30	20	24	22	17
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	1	2	0	0	1	0
--B-"C" CELL ADENOMA		5	7	5	6	3	6	11	4
--M-"C" CELL CARCINOMA		1	4	1	0	1	1	0	1
--B-FOLLICULAR CELL ADENOMA		2	5	0	2	1	1	0	2
--M-FOLLICULAR CELL CARCINOMA		0	1	0	0	0	1	0	0
--HYPERPLASIA, "C" CELL		4	3	7	5	11	11	13	6
--HYPERPLASIA, FOLLICULAR CELL		1	0	1	0	0	0	2	1
--CYST, ULTIMOBRANCHIAL		22	17	13	17	19	13	16	22
--FOLLICULAR ECTASIS		18	15	17	14	25	20	22	32
--FOLLICLE, CYST		4	7	5	4	0	0	3	0
--PIGMENT, FOLLICULAR CELL		1	0	0	0	0	0	0	0
PARATHYROID (PT)	NUMBER EXAMINED:	59	63	60	60	58	65	60	60
	NOT REMARKABLE:	50	50	51	50	54	61	54	53
--B-ADENOMA		0	0	1	0	0	0	0	1
--HYPERPLASIA		6	7	8	6	1	2	5	3
--FIBROSIS		4	6	0	4	3	3	1	5

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
ESOPHAGUS (ES)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	62	63	59	61	63	65	63	65
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	0	2	0	0	0	0
--HEMORRHAGE		0	0	1	0	0	0	0	0
--PERFORATION		0	1	1	0	0	0	0	0
--FOREIGN BODY		0	0	0	2	0	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	0	1	1	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	1	1	0	0	1	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	1	0	0	0
--CHRONIC INFLAMMATION, TUNICA MUSCULARIS		1	2	3	0	1	0	1	0
TRACHEA (TR)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	65	65	65	63	65	65	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	2	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	0	1	1
LUNG (LU)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	9	18	16	9	19	19	31	22
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		5	1	3	8	0	0	0	2
--N-MAMMARY CARCINOMA		0	0	0	0	1	0	0	0
--N-CARCINOSARCOMA, MAMMARY		0	0	0	0	0	1	0	0
--N-PHEOCHROMOCYTOMA		0	1	0	0	0	0	0	1
--N-CARCINOMA, ENDOMETRIUM,UTERUS		0	0	0	0	0	0	0	1
--I-OSTEOSARCOMA		1	0	0	0	0	0	0	0
--ALVEOLUS/BRONCHUS EPITHELIAL HYPERPLASIA		0	0	0	1	1	0	0	0
--MINERALIZATION, VASCULAR		26	33	27	34	17	31	21	15
--BONE SPICULE		2	1	0	0	0	0	0	0
--ALVEOLAR FOAMY MACROPHAGES		14	6	6	8	12	7	8	9
--CHRONIC INFLAMMATION, FOCAL		7	6	8	8	6	2	0	8
--INFLAMMATION, GRANULOMATOUS		5	1	2	4	9	7	1	2

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

		-- NUMBER-OF-ANIMALS-AFFECTED --							
TABLE INCLUDES:									
SEX=ALL;GROUP=ALL;WEEKS=1-106	SEX:	-------MALE-------				------FEMALE------			
DEATH=ALL;FIND=ALL;SUBSET=ALL									
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
LUNG (LU)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	9	18	16	9	19	19	31	22
--INFLAMMATION, CHRONIC ACTIVE		2	2	3	0	2	3	0	1
--PLEURITIS		0	0	0	2	0	0	0	0
--EDEMA		2	1	1	0	0	0	2	1
--VASCULITIS		0	0	0	1	0	0	0	0
--PERIBRONCHIAL, INFILTRATION, LYMPHOID		1	0	0	1	0	0	0	0
--CONGESTION		16	14	23	13	12	13	14	15
--HEMORRHAGE		1	2	0	3	2	1	1	6
HEART (HT)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	3	2	10	5	19	19	17	11
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	2	2	0	0	1	0
--M-ENDOCARDIAL SCHWANNOMA		0	0	0	0	0	0	1	0
--N-CARCINOSARCOMA, MAMMARY		0	0	0	0	0	1	0	0
--ENDOCARDIAL HYPERPLASIA		1	1	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	1	0	0	0	0
--CARDIOMYOPATHY, DEGENERATIVE		59	63	53	55	46	45	47	54
--PERICARDITIS		0	0	0	1	0	0	0	0
--THROMBOSIS, ATRIAL		1	0	0	0	0	0	0	0
--ARTERITIS/PERIARTERITIS		0	0	0	0	0	0	0	1
--FIBRINOID NECROSIS, ARTERIAL WALL		0	0	0	0	0	0	0	1
--MINERALIZATION		0	1	1	0	1	0	2	0
--VESSEL, MINERALIZATION		0	2	1	5	1	1	1	1
--MEDIAL HYPERTROPHY, INTRAMURIAL ARTERIES		0	1	0	0	0	0	0	0
SPLEEN (SP)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	4	2	1	1	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	2	8	0	0	1	1
--M-HEMANGIOSARCOMA		0	0	1	0	0	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

		-- NUMBER-OF-ANIMALS-AFFECTED --							
TABLE INCLUDES:									
SEX=ALL;GROUP=ALL;WEEKS=1-106	SEX:	-------MALE-------				------FEMALE------			
DEATH=ALL;FIND=ALL;SUBSET=ALL									
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
SPLEEN (SP)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	4	2	1	1	0	0	0	0
--I-FIBROSARCOMA		0	0	0	1	0	0	0	0
--EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		0	4	2	6	10	4	6	6
--PIGMENT, INCREASED		59	62	61	57	64	65	65	64
--HYPERPLASIA, RETICULOENDOTHELIAL		0	1	1	1	0	0	1	1
--HYPERPLASIA, LYMPHOID		0	1	0	0	0	0	0	0
--DEPLETION, LYMPHOID		8	6	10	6	12	9	10	6
--LEUKOCYTOSIS		0	0	0	1	0	0	0	0
--CYST		0	0	0	0	0	0	0	1
LIVER (LI)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	1	0	4	0	6	4	3	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		5	1	3	8	0	1	2	3
--B-ADENOMA, HEPATOCELLULAR		1	1	0	3	0	0	1	5
--M-CARCINOMA, HEPATOCELLULAR		1	3	1	2	0	0	0	0
--N-OSTEOSARCOMA		0	1	0	0	0	0	0	0
--CHOLANGIOFIBROSIS		0	0	0	2	0	1	0	1
--HYPERTROPHY, HEPATOCELLULAR, CENTRILOBULAR		4	9	5	9	4	3	4	3
--CONGESTION		13	7	12	12	7	6	8	8
--HEMORRHAGE		1	0	0	0	1	0	0	0
--ANGIECTASIS		1	1	2	2	0	0	1	0
--EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		5	8	5	4	10	9	12	8
--VACUOLIZATION, DIFFUSE		1	0	1	0	0	0	0	0
--VACUOLIZATION, PORTAL/PERIPORTAL		15	14	14	19	15	11	5	14
--VACUOLIZATION, CENTRILOBULAR		2	2	2	1	2	0	2	0
--VACUOLIZATION, FOCAL		1	1	0	0	0	1	4	2
--BILE DUCT, HYPERPLASIA		36	38	27	20	21	25	20	15
--BILE DUCT, INFLAMMATION, CHRONIC		39	46	41	31	24	24	23	19
--BILE DUCT, ECTASIS		17	20	14	11	3	6	5	5
--BILE DUCT, FIBROSIS		26	29	22	13	8	11	8	5

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
LIVER (LI)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	1	0	4	0	6	4	3	2
--BILE STASIS		2	0	0	0	0	0	0	0
--FIBROSIS		0	0	0	1	0	0	0	1
--INFLAMMATION, CHRONIC, FOCAL		18	18	12	10	15	12	14	12
--INFLAMMATION, GRANULOMATOUS		0	0	0	0	0	0	0	1
--NECROSIS		1	0	3	1	3	4	4	4
--HEPATOCELLULAR NECROSIS, CENTRILOBULAR		0	0	0	0	0	2	0	0
--HEPATOCELLULAR DEGENERATION, CENTRILOBULAR		0	0	0	0	0	0	1	1
--PIGMENT, SINUSOIDAL CELL		6	7	6	6	17	22	20	11
--CELLULAR ALTERATION, CLEAR		3	6	4	3	8	10	5	13
--CELLULAR ALTERATION, BASOPHILIC		5	8	2	4	4	5	5	19
--CELLULAR ALTERATION, EOSINOPHILIC		7	2	4	8	12	8	20	15
--OSSEOUS METAPLASIA		1	0	0	0	0	0	0	0
--HEPATOCYTES, KARYOMEGALLY		0	0	1	0	0	0	0	0
--THROMBUS		1	1	0	3	0	0	0	0
--SPONGIOSIS HEPATIS		4	6	5	1	0	0	0	0
KIDNEY (KD)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	1	2	2	3	1	4	1	8
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		4	1	1	6	0	0	1	0
--B-LIPOMA		1	0	0	0	0	0	0	0
--M-NEPHROBLASTOMA		0	0	0	0	0	0	1	0
--NEPHROPATHY, CHRONIC PROGRESSIVE		59	61	54	53	41	35	33	30
--CYST		6	7	9	3	1	1	2	1
--PELVIS, DILATATION		1	2	4	1	2	3	3	4
--PELVIS, CALCULUS		25	26	14	1	57	55	56	40
--INFLAMMATION, CHRONIC ACTIVE		2	0	1	1	0	0	0	0
--INFLAMMATION, CHRONIC		2	0	3	0	0	1	0	0
--BACTERIAL COLONIES, MEDULLARY TUBULE		1	0	0	0	0	0	0	0
--MINERALIZATION, VASCULAR		0	0	0	1	0	1	0	0
*** CONTINUED ON NEXT PAGE ***									

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
KIDNEY (KD)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	1	2	2	3	1	4	1	8
--TUBULE, MINERALIZATION		7	14	11	12	37	26	27	29
--TUBULE, REGENERATION		1	0	1	1	0	2	0	0
--TUBULE, PIGMENT		1	0	0	0	0	0	0	0
--CHRONIC ACTIVE INFLAMMATION, PELVIS		4	7	5	1	4	3	2	6
--TRANSITIONAL CELL HYPERPLASIA		2	8	1	3	6	1	7	4
--SUPPURATIVE PYELONEPHRITIS		0	1	2	2	0	0	0	0
--TUBULE, PROTEINACEOUS CASTS		0	0	1	0	0	1	0	0
--TUBULE, PROTEIN RESORPTION DROPLETS		2	0	4	5	0	1	0	1
--PAPILLA, NECROSIS		0	1	0	0	0	0	0	0
--THROMBOSIS		0	0	1	0	1	0	0	0
--ANGIECTASIS		0	0	0	0	1	1	0	0
--HEMORRHAGE		0	0	0	0	0	1	0	0
--ARTERITIS/PERIARTERITIS		0	0	0	0	0	0	1	0
--VACUOLIZATION, PAPILLARY EPITHELIUM		0	1	0	0	0	0	0	0
STOMACH, NONGL (SU)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	57	12	3	1	59	20	5	4
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	1	1	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		1	1	5	6	0	0	1	0
--M-SQUAMOUS CELL CARCINOMA		1	0	1	1	0	1	0	1
--HEMORRHAGE		0	2	1	2	0	0	0	1
--HYPERPLASIA		3	51	60	57	4	44	60	58
--HYPERKERATOSIS		4	17	21	46	3	19	31	27
--EDEMA		2	14	23	43	4	16	26	33
--KERATINIC CYST		1	2	7	11	0	0	1	10
--ULCER		2	0	0	0	4	0	0	1
--INFLAMMATION, CHRONIC		0	0	1	9	0	0	4	1
--INFLAMMATION, CHRONIC ACTIVE		3	1	2	10	4	2	2	4

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
STOMACH, GL (ST)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	9	9	9	9	6	11	5	8
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	0	3	0	0	0	0
--I-FIBROSARCOMA		0	0	0	0	0	1	0	0
--HYPERPLASIA		6	6	6	5	1	1	3	3
--CONGESTION		2	2	3	1	2	1	0	3
--DILATATION, MUCOSAL GLAND		53	50	49	50	56	53	59	54
--EROSION/ULCER		6	2	7	5	6	2	1	2
--EDEMA		0	0	0	1	0	0	0	0
--NECROSIS		0	1	0	3	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		3	1	2	3	0	0	0	0
--ARTERITIS/PERIARTERITIS		0	0	0	0	0	0	0	1
--FIBRINOID NECROSIS, ARTERIAL WALL		0	0	0	0	0	0	0	1
--MINERALIZATION		0	1	2	0	0	1	0	1
--ECTOPIC STOMACH		0	1	0	0	0	1	0	0
DUODENUM (DU)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	64	64	63	63	65	64	65	65
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	2	0	0	0	0
--CONGESTION		0	1	1	0	0	1	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	1	0	0
JEJUNUM (JE)	NUMBER EXAMINED:	60	64	61	65	63	64	64	64
	NOT REMARKABLE:	59	64	60	63	63	63	61	63
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	2	0	0	0	1
--CONGESTION		1	0	0	0	0	1	2	0
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--VILLOUS ATROPHY, FOCAL		0	0	0	0	0	0	1	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
ILEUM (IL)	NUMBER EXAMINED:	65	65	64	65	65	65	65	65
	NOT REMARKABLE:	64	65	64	64	63	65	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	0
--CONGESTION		1	0	0	0	1	0	1	0
--INFLAMMATION, CHRONIC		1	0	0	0	1	0	0	0
--PARASITISM		0	0	0	0	0	0	0	1
PANCREAS (PA)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	39	40	51	48	47	54	53	45
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	1	5	0	0	1	0
--B-ADENOMA, ACINAR CELL		0	1	1	0	0	0	0	0
--M-CARCINOMA, ACINAR CELL		0	1	0	2	0	0	0	1
--B-ADENOMA, ISLET CELL		4	3	1	1	3	1	0	0
--M-CARCINOMA, ISLET CELL		0	1	1	0	2	0	0	0
--N-GRANULOSA/THECA CELL TUMOR		0	0	0	0	0	0	0	1
--N-OSTEOSARCOMA		0	1	0	0	0	0	0	0
--I-FIBROSARCOMA		0	0	0	1	0	0	0	0
--HYPERTROPHY/HYPERPLASIA		0	5	2	0	0	2	1	2
--ISLET CELL, HYPERPLASIA		1	0	1	0	3	0	1	2
--VACUOLIZATION		0	1	0	0	0	0	0	1
--ATROPHY, FOCAL, ACINAR		11	11	7	8	6	4	4	13
--ARTERITIS/PERIARTERITIS		2	2	0	0	1	2	1	0
--FIBRINOID NECROSIS, VASCULAR WALL		1	0	0	0	0	1	1	0
--HEPATOCYTIC FOCUS		0	0	0	0	0	0	0	2
--BASOPHILIC FOCUS		4	1	3	0	3	0	2	1
--INFLAMMATION, CHRONIC		4	1	0	2	1	1	2	0
--MICROGRANULOMA		0	0	0	0	0	1	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
CECUM (CE)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	61	64	63	59	61	62	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	1
--B-LEIOMYOMA		0	1	0	1	0	0	0	0
--CONGESTION		2	0	0	0	0	2	0	0
--HEMORRHAGE		1	0	1	2	0	1	0	0
--NECROSIS		0	0	1	2	0	0	0	0
--ULCERATION		1	0	1	4	0	1	0	0
--EROSION		0	0	0	0	1	0	0	0
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		2	0	1	4	3	0	1	0
--ARTERITIS/PERIARTERITIS		0	0	1	0	0	0	0	0
COLON (CO)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	57	61	58	52	57	53	50	62
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	2	0	0	0	0
--CONGESTION		1	0	0	0	0	1	1	0
--DILATATION		5	4	4	10	8	9	13	3
--PARASITISM		1	0	1	0	1	2	1	0
--ULCERATION		0	0	0	1	0	0	0	0
--NECROSIS		0	0	0	1	0	0	0	0
--CYST, SUBMUCOSAL		0	0	1	0	0	0	0	0
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	2	0	0	0	0
--ARTERITIS/PERIARTERITIS		0	0	1	0	0	0	0	0
--FIBRINOID NECROSIS, VASCULAR WALL		0	0	1	0	0	0	0	0
RECTUM (RE)	NUMBER EXAMINED:	65	65	65	65	64	65	65	65
	NOT REMARKABLE:	55	50	52	59	54	54	51	55
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
RECTUM (RE)	NUMBER EXAMINED:	65	65	65	65	64	65	65	65
	NOT REMARKABLE:	55	50	52	59	54	54	51	55
--PARASITISM		7	11	10	2	3	5	3	7
--DILATATION		3	4	2	1	6	6	12	3
--ULCERATION		0	0	0	1	0	0	0	0
--NECROSIS		0	0	0	1	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	2	1	0	0	0
--ARTERITIS/PERIARTERITIS		0	0	1	0	0	0	0	0
--FIBRINOID NECROSIS, VASCULAR WALL		0	0	1	0	0	0	0	0
LN, MESENTERIC (MS)	NUMBER EXAMINED:	65	64	64	64	65	65	65	65
	NOT REMARKABLE:	14	9	14	13	7	11	19	11
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		5	0	2	8	0	0	0	3
--B-HEMANGIOMA		1	0	0	0	0	0	0	0
--M-HEMANGIOSARCOMA		0	3	0	0	0	0	0	0
--MACROPHAGES, PIGMENTED		10	12	11	21	16	22	16	21
--FOCI OF PALE MACROPHAGES		46	48	42	32	49	39	37	41
--HYPERPLASIA, LYMPHOID		1	2	1	0	0	0	0	2
--CONGESTION/ERYTHROPHAGOCYTOSIS		7	5	5	10	1	7	4	2
--LYMPHANGIECTASIS		0	0	0	1	1	1	0	0
--EDEMA		0	0	0	0	0	0	1	1
--ANGIECTASIS		0	0	0	1	0	0	0	0
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	65	65	65	65	64	65	65	65
	NOT REMARKABLE:	34	39	36	36	25	31	27	35
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	0	6	0	0	0	1
--MACROPHAGES, PIGMENTED		17	18	23	12	31	29	27	23
--FOCI OF PALE MACROPHAGES		3	2	0	0	0	1	1	0
--CONGESTION/ERYTHROPHAGOCYTOSIS		4	3	4	6	10	3	6	6
--LEUKOCYTOSIS		1	0	0	0	0	1	1	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	65	65	65	65	64	65	65	65
	NOT REMARKABLE:	34	39	36	36	25	31	27	35
--HYPERPLASIA, LYMPHOID		9	5	4	12	6	7	7	8
--LYMPHANGIECTASIS		1	3	2	2	1	0	3	0
SALIV GL, MANDIB (SG)	NUMBER EXAMINED:	65	65	65	65	65	65	65	64
	NOT REMARKABLE:	64	65	63	63	64	65	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	2	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	1	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	0	1	0
--VACUOLIZATION		0	0	1	0	1	0	0	0
THYMUS (TH)	NUMBER EXAMINED:	55	56	55	60	58	61	60	60
	NOT REMARKABLE:	20	31	21	21	27	24	22	29
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	1	5	0	0	1	0
--M-THYMOMA		1	0	0	1	0	0	0	0
--CONGESTION		1	1	3	1	0	1	1	0
--DEPLETION, LYMPHOID		27	22	28	28	24	29	26	21
--CYST		2	1	2	6	12	17	22	14
--HYPERPLASIA, MEDULLARY EPITHELIAL		2	1	0	0	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0	0	1	0
AORTA, THORACIC (AO)	NUMBER EXAMINED:	64	65	65	65	64	65	65	65
	NOT REMARKABLE:	63	64	64	61	63	64	65	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	2	0	0	0	0
--MEDIA, MINERALIZATION		1	1	1	2	1	1	0	1

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
EYE (EY)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	64	62	57	63	64	64	62	63
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	2	1	0	0	0	1
--KERATITIS		0	0	0	0	1	0	0	0
--LENS, DEGENERATION		0	1	3	0	0	0	2	0
--ULCER, CORNEA		0	0	0	0	1	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	1	0	0	0	0	0
--HEMORRHAGE, PERIORBITAL		0	0	0	0	0	1	0	0
--MINERALIZATION, SCLERA		0	1	0	0	0	0	0	1
--ATROPHY, RETINA		0	1	2	0	0	0	1	0
HARDERIAN GLAND (HG)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	21	32	37	36	22	38	31	30
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	2	0	0	0	0	1
--INFLAMMATION, CHRONIC		43	33	26	29	43	27	34	34
LACRIMAL GL, EX (EO)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	31	26	31	36	34	34	41	33
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	0	0	0
--VACUOLAR DEGENERATION/INFLAMMATION		33	39	34	28	31	31	24	32
MUSCLE, SKELETAL (SM)	NUMBER EXAMINED:	65	65	65	65	65	64	65	65
	NOT REMARKABLE:	64	62	65	62	63	62	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	1	0	0	1	0
--INFLAMMATION, CHRONIC		0	2	0	0	1	0	0	0
--DEGENERATION		0	1	0	2	1	2	0	1

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	62	65	65	65	65	65	65	64
	NOT REMARKABLE:	61	64	65	64	65	65	64	64
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	1	0	0	1	0
--MINERALIZATION		0	1	0	0	0	0	0	0
AUDITORY SEB GL (AS)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	65	65	65	65	65	65	65	65
TONGUE (TO)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	62	65	65	63	65	65	65	65
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	1	0	0	0	0
--ARTERITIS/PERIARTERITIS		2	0	0	0	0	0	0	0
--FIBRINOID NECROSIS, VASCULAR WALL		1	0	0	0	0	0	0	0
TESTIS (TE)	NUMBER EXAMINED:	65	65	65	65	0	0	0	0
	NOT REMARKABLE:	34	32	37	34	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0	0	0	0
--B-BENIGN INTERSTITIAL CELL TUMOR		2	4	1	10	0	0	0	0
--HYPERPLASIA, INTERSTITIAL CELL		9	4	4	5	0	0	0	0
--DEGENERATION		24	25	20	20	0	0	0	0
--HYPOSPERMIA		9	8	7	8	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	1	0	0	0	0
--EDEMA		0	4	1	2	0	0	0	0
--MINERALIZATION		5	4	6	3	0	0	0	0
--MINERALIZATION, VASCULAR WALL		0	0	2	4	0	0	0	0
--MULTINUCLEATED CELLS		2	1	0	3	0	0	0	0
--ARTERITIS/PERIARTERITIS		3	5	1	1	0	0	0	0
--FIBRINOID NECROSIS, ARTERIAL WALL		3	3	0	0	0	0	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
EPIDIDYMIS (EP)	NUMBER EXAMINED:	65	65	65	65	0	0	0	0
	NOT REMARKABLE:	54	51	51	54	0	0	0	0
--HYPOSPERMIA		9	7	7	9	0	0	0	0
--INFLAMMATORY INFILTRATE, PERIVASCULAR		2	7	7	2	0	0	0	0
--DEGENERATION		0	0	0	1	0	0	0	0
PROSTATE (PR)	NUMBER EXAMINED:	65	65	65	65	0	0	0	0
	NOT REMARKABLE:	9	10	12	11	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		4	0	1	3	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		53	55	52	51	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	1	0	0	0	0	0
--ABSCESS		0	0	0	1	0	0	0	0
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	65	65	65	65	0	0	0	0
	NOT REMARKABLE:	50	53	47	44	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	1	2	0	0	0	0
--I-FIBROSARCOMA		0	0	0	1	0	0	0	0
--DISTENTION		0	0	1	2	0	0	0	0
--DECREASED SECRETION		11	7	11	13	0	0	0	0
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		1	5	8	5	0	0	0	0
--FIBROSIS		1	0	0	0	0	0	0	0
URINARY BLADDER (UB)	NUMBER EXAMINED:	65	65	65	65	64	65	64	65
	NOT REMARKABLE:	57	56	57	57	62	63	60	60
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	1	0	0	1	1
--B-TRANSITIONAL CELL PAPILLOMA		0	0	0	0	0	0	1	0
--M-LEIOMYOSARCOMA		0	0	0	0	1	0	0	0
--HYPERPLASIA		4	3	5	5	1	1	2	3

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
URINARY BLADDER (UB)	NUMBER EXAMINED:	65	65	65	65	64	65	64	65
	NOT REMARKABLE:	57	56	57	57	62	63	60	60
--HEMORRHAGE		0	0	0	0	0	0	0	1
--FIBROSIS, FOCAL		0	0	0	0	0	1	0	0
--INTRALUMINAL PROTEINACEOUS MATERIAL		2	5	2	1	0	0	0	0
--INFLAMMATION, CHRONIC		2	1	0	1	1	1	2	0
--INFLAMMATION, CHRONIC ACTIVE		4	3	2	2	0	0	0	2
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	65	65	65	65
	NOT REMARKABLE:	0	0	0	0	26	34	35	27
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1
--B-TUBULAR ADENOMA, SERTOLIFORM		0	0	0	0	0	0	0	1
--B-GRANULOSA/THECA CELL TUMOR		0	0	0	0	0	0	1	0
--M-MALIGNANT GRANULOSA/THECA CELL TUMOR		0	0	0	0	1	0	0	3
--HYPERPLASIA, TUBULAR		0	0	0	0	29	18	24	21
--FOLLICLE, CYST		0	0	0	0	12	15	6	14
--BURSA, CYST		0	0	0	0	3	0	2	2
--FIBROSIS		0	0	0	0	0	1	0	0
--UNILATERALLY EXAMINED		0	0	0	0	2	1	2	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	65	65	65	65
	NOT REMARKABLE:	0	0	0	0	55	53	55	43
--B-ENDOMETRIAL STROMAL POLYP		0	0	0	0	2	0	1	4
--M-CARCINOMA		0	0	0	0	0	0	0	1
--M-SCHWANNOMA		0	0	0	0	0	1	0	0
--I-PANCREATIC ACINAR CARCINOMA		0	0	0	0	0	0	0	1
--HYPERPLASIA		0	0	0	0	0	1	0	0
--THROMBOSIS		0	0	0	0	0	0	0	1
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	2	1	0	4
--DILATATION		0	0	0	0	7	6	3	14

*** CONTINUED ON NEXT PAGE ***

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	65	65	65	65
	NOT REMARKABLE:	0	0	0	0	55	53	55	43
--DILATATION, ENDOMETRIAL GLAND		0	0	0	0	4	7	9	7
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	65	65	64	65
	NOT REMARKABLE:	0	0	0	0	63	64	62	63
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1
--B-GRANULAR CELL TUMOR		0	0	0	0	1	0	0	0
--M-LEIOMYOSARCOMA		0	0	0	0	0	0	1	1
--PROLAPSED		0	0	0	0	0	0	1	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	1	1	0
--STROMAL PROLIFERATION		0	0	0	0	1	0	0	0
MAMMARY, FEMALE (MF)	NUMBER EXAMINED:	0	0	0	0	65	65	65	63
	NOT REMARKABLE:	0	0	0	0	27	31	33	34
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0
--B-FIBROMA		0	0	0	0	0	0	0	1
--M-FIBROSARCOMA		0	0	0	0	0	0	0	1
--B-FIBROADENOMA		0	0	0	0	14	18	14	15
--B-FIBROADENOMA, MULTIPLE		0	0	0	0	2	1	4	4
--M-CARCINOSARCOMA		0	0	0	0	0	1	0	0
--M-CARCINOMA		0	0	0	0	12	6	5	4
--M-CARCINOMA, MULTIPLE		0	0	0	0	1	2	1	0
--HYPERPLASIA		0	0	0	0	12	8	7	5
--GALACTOCELE		0	0	0	0	17	13	14	7
--FIBROSIS		0	0	0	0	1	0	0	0
--ABSCESS		0	0	0	0	1	0	1	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
SKIN (SK)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	63	64	64	63	64	65	65	63
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	1	1	1	0	0	0	0
--ACANTHOSIS		0	0	0	1	0	0	0	0
--ULCERATION		0	0	0	0	1	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	1	0	0	0
--NECROSIS		0	0	0	0	1	0	0	0
--DERMAL INCLUSION CYST		0	0	0	0	0	0	0	2
MARROW, STERNUM (SE)	NUMBER EXAMINED:	65	65	65	64	65	65	65	64
	NOT REMARKABLE:	26	25	19	21	18	21	18	11
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	1	3	0	0	1	3
--HYPERPLASIA, MYELOID		3	3	4	5	6	5	3	5
--INCREASED ADIPOCYTES		35	38	41	34	41	39	43	45
--HYPEROSTOSIS		0	0	0	1	0	0	0	0
BONE, STERNUM (SB)	NUMBER EXAMINED:	65	65	65	65	65	65	65	64
	NOT REMARKABLE:	65	65	65	65	65	65	65	64
MARROW, FEMUR (FM)	NUMBER EXAMINED:	65	65	65	65	65	63	65	64
	NOT REMARKABLE:	12	9	16	11	14	18	15	10
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		3	0	2	6	0	0	1	3
--INCREASED ADIPOCYTES		47	54	46	44	45	41	47	46
--HYPERPLASIA, MYELOID		3	3	3	4	6	4	2	5
--HYPEROSTOSIS		0	0	0	1	0	0	0	0
BONE, FEMUR (FE)	NUMBER EXAMINED:	65	65	65	65	65	63	65	64
	NOT REMARKABLE:	65	65	65	65	65	63	65	64

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
NASAL TURBINATE (NT)	NUMBER EXAMINED:	65	65	65	64	65	65	65	65
	NOT REMARKABLE:	47	49	52	39	47	47	52	57
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	2	0	0	1	1
--B-ODONTOMA		1	0	1	0	0	0	0	0
--M-FIBROSARCOMA		0	0	1	0	0	0	0	0
--I-FIBROSARCOMA		0	0	0	0	0	0	0	1
--HEMORRHAGE		0	0	0	3	0	0	0	1
--SKIN, CHRONIC ACTIVE INFLAMMATION		0	1	0	0	1	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		16	13	10	20	17	15	10	7
--ABSCESS		0	4	1	1	0	0	2	0
--ULCERATION		0	1	0	0	1	1	0	0
--FOLLICULITIS		0	0	0	0	0	2	0	0
--FOREIGN BODY		0	0	0	0	0	1	0	0
^DEATH COMMENT (DC)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--SCHEDULED SACRIFICE		32	43	34	33	21	23	26	35
--UNDETERMINED		6	0	6	2	3	1	5	2
--ACCIDENTAL		0	0	2	0	0	0	0	0
--GAVAGE ERROR		0	1	1	2	0	0	1	0
--INFLAMMATION		8	0	1	4	1	3	0	1
--NEPHROPATHY		0	0	1	0	0	0	0	0
--SUPPURATIVE PYELONEPHRITIS		0	0	1	1	0	0	0	0
--ABSCESS, BRAIN		0	0	0	0	0	1	0	0
--PITUITARY TUMOR		9	12	11	10	26	29	21	11
--HEMATOPOIETIC NEOPLASIA		6	1	4	7	0	1	2	3
--ASTROCYTOMA		0	0	0	1	0	0	0	1
--OLIGODENDROGLIOMA		0	1	0	0	0	0	0	0
--OSTEOSARCOMA		1	1	0	1	0	0	1	0
--AMELANOTIC MELANOMA		0	0	1	0	0	0	0	0
--HEMANGIOSARCOMA		0	0	0	0	1	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-106
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
^DEATH COMMENT (DC)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--NEPHROBLASTOMA		0	0	0	0	0	0	1	0
--GRANULAR CELL TUMOR		0	0	0	0	0	1	0	0
--GRANULOSA/THECA TUMOR		0	0	0	0	0	0	0	2
--CARCINOMA, MAMMARY		0	0	0	0	10	2	3	3
--CARCINOMA, SQUAMOUS CELL		1	1	1	1	0	1	1	1
--CARCINOMA, ACINAR CELL		0	0	0	1	0	0	0	1
--CARCINOMA, UTERUS		0	0	0	0	0	0	0	1
--CARCINOMA, ISLET CELL		0	1	0	0	0	0	0	0
--CARCINOMA, HEPATOCELLULAR		0	0	0	1	0	0	0	0
--MAMMARY CARCINOSARCOMA		0	0	0	0	0	1	0	0
--MAMMARY FIBROADENOMA		0	0	0	0	3	2	1	1
--ENDOCARDIAL SCHWANNOMA		0	0	0	0	0	0	1	0
--SCHWANNOMA		0	0	0	0	0	0	1	1
--FIBROMA		2	1	0	0	0	0	0	0
--FIBROSARCOMA		0	1	2	1	0	0	0	2
--LEIOMYOSARCOMA		0	0	0	0	0	0	1	0
--SARCOMA, NOS		0	1	0	0	0	0	0	0
--MYXOSARCOMA		0	1	0	0	0	0	0	0
HEMATO NEOPLASIA (HN)	NUMBER EXAMINED:	65	65	65	65	65	65	65	65
	NOT REMARKABLE:	58	64	61	54	65	64	63	61
--M-SARCOMA, HISTIOCYTIC		6	1	4	10	0	1	1	4
--M-LYMPHOMA		0	0	0	1	0	0	1	0
--M-LEUKEMIA, GRANULOCYTIC		1	0	0	0	0	0	0	0
ZYMBAL'S GLAND (ZG)	NUMBER EXAMINED:	57	63	62	61	61	62	63	64
	NOT REMARKABLE:	45	61	57	56	53	53	57	62
--B-ADENOMA		0	0	0	0	1	0	0	0

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-106
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
ZYMBAL'S GLAND (ZG)	NUMBER EXAMINED:	57	63	62	61	61	62	63	64
	NOT REMARKABLE:	45	61	57	56	53	53	57	62
--M-SQUAMOUS CELL CARCINOMA		0	0	1	2	0	0	0	0
--DUCT ECTASIS		1	0	2	0	2	3	1	1
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	0	1	1
--INFLAMMATION, SUPPURATIVE		0	1	0	1	0	1	0	0
--UNILATERALLY EXAMINED		10	1	2	2	6	5	5	0
SKIN, OTHER (SS)	NUMBER EXAMINED:	45	43	32	41	14	15	12	24
	NOT REMARKABLE:	0	0	1	1	3	0	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	2	0	0	0	0
--B-FIBROMA		2	0	0	0	0	0	0	0
--B-NEUROFIBROMA		0	0	0	0	0	1	0	0
--B-KERATOACANTHOMA		4	3	2	3	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	1	0	0	0	0
--B-TRICHOEPITHELIOMA		0	0	1	0	0	0	0	0
--M-SARCOMA, NOS		0	1	0	0	0	0	0	0
--M-SCHWANNOMA		0	0	0	0	0	0	0	1
--M-MYXOSARCOMA		0	1	0	0	0	0	0	0
--M-HEMANGIOSARCOMA		0	0	0	0	1	0	0	0
--M-AMELANOTIC MELANOMA		0	0	1	0	0	0	0	0
--M-SQUAMOUS CELL CARCINOMA		0	0	0	1	0	0	0	0
--M-BASAL CELL CARCINOMA		0	1	0	0	0	0	0	0
--ACANTHOSIS		14	12	6	12	1	4	1	0
--ULCERATION		11	1	5	12	2	5	4	5
--INFLAMMATION, CHRONIC ACTIVE		17	12	14	23	6	7	5	10
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0	1	0	0
--ABSCESS		4	9	3	9	3	2	0	1
--NECROTIC CELL DEBRIS, EPIDERMAL SURFACE		16	8	10	17	6	8	7	9
--HYPERKERATOSIS		2	4	3	2	2	1	1	2

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
SKIN, OTHER (SS)	NUMBER EXAMINED:	45	43	32	41	14	15	12	24
	NOT REMARKABLE:	0	0	1	1	3	0	1	2
--NECROSIS		7	0	3	2	1	2	0	0
--PODODERMATITIS		3	4	2	5	2	0	1	2
--ULCERATIVE PODODERMATITIS		15	14	11	10	1	1	3	5
--FIBROSIS		1	0	0	0	0	0	0	0
--DERMAL INCLUSION CYST		2	9	8	4	0	0	2	2
--HEMORRHAGE		0	0	0	1	0	0	0	0
CAVITY, THORACIC (TA)	NUMBER EXAMINED:	1	3	3	3	0	2	3	0
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	2	2	0	0	1	0
--B-FIBROMA		0	1	0	0	0	0	0	0
--B-LIPOMA		0	0	0	0	0	1	0	0
--M-OSTEOSARCOMA		1	0	0	0	0	0	0	0
--M-MALIGNANT HIBERNOMA		0	0	0	0	0	0	1	0
--M-CHORDOMA		0	0	0	0	0	0	1	0
--I-FIBROSARCOMA		0	0	0	0	0	1	0	0
--ARTERITIS		0	1	0	0	0	0	0	0
--MINERALIZATION, VASCULAR WALL		0	0	1	1	0	0	0	0
LN, OTHER (LN)	NUMBER EXAMINED:	27	31	20	21	37	39	27	36
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	1	4	0	0	0	2
--N-OSTEOSARCOMA		0	1	0	0	0	0	0	0
--HYPERPLASIA, LYMPHOID		0	0	1	0	0	0	0	0
--MACROPHAGES, PIGMENTED		24	24	18	16	37	38	27	34
--CONGESTION/ERYTHROPHAGOCYTOSIS		17	9	8	6	20	20	12	13
--LYMPHANGIECTASIS		7	7	3	1	2	2	1	2
--AMYLOIDOSIS		0	0	0	0	0	1	0	0

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
CAVITY, ABDOM (PC)	NUMBER EXAMINED:	3	3	2	6	0	0	1	3
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		2	0	1	2	0	0	0	1
--B-LIPOMA		0	0	0	2	0	0	1	0
--M-FIBROSARCOMA		0	0	0	1	0	0	0	0
--M-OSTEOSARCOMA		0	1	0	0	0	0	0	0
--NECROSIS, FAT		0	0	0	1	0	0	0	0
--ARTERITIS/PERIARTERITIS		1	1	1	0	0	0	0	2
--FIBRINOID NECROSIS, ARTERIAL WALL		0	0	0	0	0	0	0	1
SUBCUTANEOUS TIS (SQ)	NUMBER EXAMINED:	5	4	6	6	0	2	3	0
	NOT REMARKABLE:	2	2	1	3	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	1	1	0	0	1	0
--B-FIBROMA		2	0	0	3	0	0	0	0
--M-SQUAMOUS CELL CARCINOMA		0	0	1	0	0	0	1	0
--M-SCHWANNOMA		0	0	0	0	0	0	1	0
--M-FIBROSARCOMA		0	1	2	0	0	1	0	0
--INFLAMMATION, CHRONIC		1	0	0	0	0	0	0	0
--ABSCESS		0	0	1	0	0	0	0	0
PREPUTIAL GLAND (PG)	NUMBER EXAMINED:	2	2	0	0	0	0	0	0
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		2	1	0	0	0	0	0	0
--ECTASIS, DUCT		1	0	0	0	0	0	0	0
HEAD, CORONAL (HC)	NUMBER EXAMINED:	0	1	0	2	0	0	1	1
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--M-OSTEOSARCOMA		0	0	0	1	0	0	1	0
--M-FIBROSARCOMA		0	0	0	0	0	0	0	1

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TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
*** FROM PREVIOUS PAGE ***									
HEAD, CORONAL (HC)	NUMBER EXAMINED:	0	1	0	2	0	0	1	1
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	1	0	0	0	0
URETER (UE)	NUMBER EXAMINED:	1	1	1	0	0	0	0	0
	NOT REMARKABLE:	0	1	0	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		1	0	1	0	0	0	0	0
--METAPLASIA		0	0	1	0	0	0	0	0
--ULCERATION		1	0	0	0	0	0	0	0
PENIS (PE)	NUMBER EXAMINED:	0	1	1	2	0	0	0	0
	NOT REMARKABLE:	0	1	1	0	0	0	0	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	2	0	0	0	0
MAMMARY, MALE (MM)	NUMBER EXAMINED:	1	2	6	3	0	0	0	0
	NOT REMARKABLE:	1	1	0	0	0	0	0	0
--GALACTOCELE		0	1	6	1	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	0	0	0
--HYPERPLASIA		0	0	0	1	0	0	0	0
CERVIX (CV)	NUMBER EXAMINED:	0	0	0	0	3	3	3	10
	NOT REMARKABLE:	0	0	0	0	2	2	3	9
--B-GRANULAR CELL TUMOR		0	0	0	0	0	1	0	0
--STROMAL PROLIFERATION		0	0	0	0	1	0	0	1

TABLE 13
INCIDENCE OF MICROSCOPIC OBSERVATIONS - ALL ANIMALS

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=ALL;FIND=ALL;SUBSET=ALL		-- N U M B E R - O F - A N I M A L S - A F F E C T E D --							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	65	65	65	65	65	65	65	65
URETHRA (UR)	NUMBER EXAMINED:	0	1	0	0	1	1	0	1
	NOT REMARKABLE:	0	1	0	0	0	0	0	1
--PROLIFERATION, MUSCULAR WALL		0	0	0	0	1	0	0	0
--B-HEMANGIOMA		0	0	0	0	0	1	0	0
CLITORAL GLAND (CL)	NUMBER EXAMINED:	0	0	0	0	0	0	1	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	1
--B-ADENOMA		0	0	0	0	0	0	1	0
CAVITY, ORAL (OC)	NUMBER EXAMINED:	0	1	0	0	0	0	0	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	1
--M-SQUAMOUS CELL CARCINOMA		0	1	0	0	0	0	0	0
*** END OF LIST ***									

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APPENDIX 3: Executive CAC Meeting Minutes for Dose Selection for the Carcinogenicity Studies.

EXECUTIVE CAC

Date of Meeting: November 27, 2001

COMMITTEE: JOSEPH DEGEORGE, PH. D., HFD-024, CHAIR

Joseph Contrera, Ph. D., HFD-901, Member
Jeri El Hage, Ph. D., HFD-510, Alternate Member
Jasti B Choudary, B.V.Sc., Ph. D., Supervisory Pharmacologist, HFD-180
Sushanta Chakder, Ph. D., Presenting Reviewer

Author of Draft: Sushanta Chakder, Ph. D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-year carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 59, 623
Drug Name: RU-0211
Sponsor: R-Tech Ueno (USA), Inc.

RU-0211 is a prostaglandin analog, and has been found to cause increased intestinal fluid secretion and decrease intestinal transit time in experimental animals. The sponsor is developing the compound for the —

Mouse Carcinogenicity Protocol and Dose Selection:

The sponsor proposed a 2-year carcinogenicity study with RU-0211 in B6C3F1 mice at 5, 10 and 35 µg/kg/day doses, administered by oral gavage. In the 13-week oral gavage toxicity study in mice, doses of 0, 10, 100, 1000 and 5000 µg/kg/day were used, and the MTD was between 100 and 1000 µg/kg/day. This was based on the observed diarrhea and the histopathological changes in the stomach (edema, acanthosis, hyperkeratosis) at 1000 and 5000 µg/kg/day doses in both sexes. There were no changes in body weights in males and females at any dose. The sponsor opted the dose selection for the carcinogenicity study on the basis of the pharmacokinetic endpoint. However, the determinations of AUC was carried out after oral administration of a single radioactive dose in both mice and humans and the total plasma radioactivities were determined by liquid scintillation counting. The method did not identify whether the total radioactivity was due to the parent compound and/or its metabolites only. In addition, the sponsor did not use the proposed

high dose in determining the AUC, and the AUC values used for dose selection were estimated values in both mice and humans. The $AUC_{0\text{-}24hr}$ values for the total radioactivity in mice after oral administration of single doses of 25 and 50 μg/kg doses were 39.6 and 66.2 ng eq.hr/ml, respectively. In the male human subjects, after a single oral dose of 72 μg (1.44 μg/kg, based on 50 kg body weight), the $AUC_{0\text{-}24hr}$ value was 2.7 ng eq.hr/ml. From this, the $AUC_{0\text{-}24hr}$ value at the proposed therapeutic dose of 48 μg/day (0.96 μg/kg, based on 50 kg body weight) was estimated as 1.8 ng eq.hr/ml. The estimated dose of RU-0211 in mice to have 25 times the human exposure was calculated as 34 μg/kg. Based on this, the sponsor decided to use 35 μg/kg as the highest dose for the 2-year carcinogenicity study in mice. The proposed mid and low doses were 10 and 5 μg/kg/day, and the estimated exposure ratios at these doses were 7.4 and 3.7, respectively. The metabolism of RU-0211 was similar in mice and humans. However, the *in vitro* plasma protein binding was slightly higher in humans as compared with that in mice. RU-0211 was not genotoxic in a battery of genotoxicity assays.

Rat Carcinogenicity Protocol and Dose Selection:

The sponsor proposed a 2-year oral gavage carcinogenicity study with RU-0211 in SD rats at 8, 16 and 65 μg/kg/day doses. In the 26-week oral gavage toxicity study in rats, doses of 0, 16, 80 and 400 μg/kg/day were used, and the MTD was 400 μg/kg/day in males and >400 μg/kg/day in females. This was based on the observation of decreased body weights in males (8.1%) and females (3.3%). Histopathological changes in the stomach (proliferation of the basal cell layer of the limiting ridge) were observed at all doses in both sexes. The sponsor opted the dose selection on the basis of the pharmacokinetic endpoint. However, the determinations of AUC was carried out after oral administration of a single radioactive dose in both rats and humans and the total plasma radioactivities were determined by liquid scintillation counting. The method did not identify whether the total radioactivity was due to the parent compound and/or its metabolites only. In addition, the sponsor did not use the proposed high dose in determining the AUC in rats, and the AUC values used for dose selection were estimated values for rats and humans. The $AUC_{0\text{-}24hr}$ values for the total radioactivity in rats after oral administration of single doses of 50 and 100 μg/kg doses were 35.5 and 76.8 ng eq.hr/ml, respectively. In the male human subjects, after a single oral dose of 72 μg (1.44 μg/kg, based on 50 kg body weight), the AUC value was 2.7 ng eq.hr/ml. From this, the $AUC_{0\text{-}24hr}$ value at the proposed therapeutic dose of 48 μg/day (0.96 μg/kg, based on 50 kg body weight) was estimated as 1.8 ng eq.hr/ml. The estimated dose of RU-0211 in rats to have 25 times the human exposure was calculated as 63 μg/kg. Based on this, the sponsor decided to use 65 μg/kg as the highest dose for the 2-year carcinogenicity study in rats that would provide >25 times the human exposure levels at the proposed therapeutic dose. The proposed mid and low doses were 16 and 8 μg/kg/day, and the estimated exposure ratios at these doses were about 11.4 and 5.7, respectively. The metabolism of RU-0211 was similar in rats and humans. The *in vitro* plasma protein binding in male humans was slightly higher than that in male rats; in females plasma protein bindings were similar. RU-0211 was not genotoxic in a battery of genotoxicity assays.

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the sponsor's high dose selections for both species based on pharmacokinetic endpoint since there was no evidence that the total radioactivity was due to RU-0211 and/or its metabolites only.
2. The committee recommended that the sponsor should conduct the 2-year mouse carcinogenicity study using 500, 200, 75 and 25 μg/kg/day doses in male and female mice, based on the estimated MTD between 100 and 1000 μg/kg/day in both males and females in the 13-week toxicity study.
3. Even though the MTD in female rats was >400 μg/kg/day, the committee recommended that the sponsor conduct the 2-year rat carcinogenicity study using 400, 100 and 20 μg/kg/day doses in both males and females, as diarrhea was observed in both sexes at 1000 μg/kg/day in the 4-week oral toxicity study.

Joseph DeGeorge, Ph. D.
Chair, Executive CAC

cc:\
/Division File, HFD-180
/JChoudary, HFD-180
/SChakder, HFD-180
CSO/PM, HFD-181
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Sushanta Chakder
12/23/2005 03:41:54 PM
PHARMACOLOGIST

Jasti Choudary
12/23/2005 03:45:44 PM
PHARMACOLOGIST

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

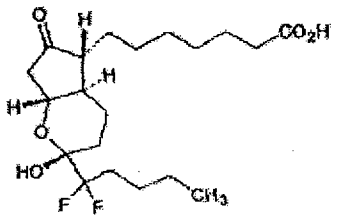
Date: November 5, 2005

From: Lynnda Reid, Ph.D.
Supervisory Pharmacologist

To: Scott Monroe, M.D. and Ronald Orleans, M.D.

Subject: NDA 21-908 - [redacted] (lubriprostone) Consult for the Division of Gastroenterology Products (HFD-180)

Background: Lubriprostone (RU-0211) is a unique PGE1 metabolite analogue being developed for the treatment of chronic idiopathic constipation.



The anticipated human dose in patients is 24 μg, administered b.i.d. In a patient weighing 60 kg, this represents a dose of 0.4 μg/kg b.i.d (0.8 μg/kg/day).

The Division of Gastroenterology Products has requested a formal consult from the Division of Reproductive and Urologic Products regarding our opinion on whether the nonclinical studies conducted to date for NDA 21-908 are adequate to determine whether lubiprostone is a potential abortifacient in humans, and if so, what is the conclusion.

Nonclinical studies reviewed by the Gastroenterology PT team on the abortifacient potential of lubiprostone for NDA 21-908 include studies in guinea pigs, rats (which may not be an appropriate model) and nonhuman primates. The PT reviewers in Gastroenterology consider the guinea pig study suggestive of abortifacient effects, the rat study irrelevant due to its lack of sensitivity to prostaglandins, and the monkey study suggestive, but potentially inadequate based on dose selection.

I concur with the conclusions of the Gastroenterology PT regarding the assessment of the studies in pregnant rats and guinea pigs. While I do agree that the monkey study was inadequate based on the unorthodox method used for dose selection method and the lack of any pharmacokinetic data to determine exposures relevant to humans, I do not see evidence suggesting that lubriprostone acts as an abortifacient in monkeys at the doses tested (see below).

In humans and primates, sensitivity of the uterus to prostaglandins increases as gestation progresses. In rats, the myometrium is refractory to prostaglandins as long as serum progesterone levels are high, while in guinea pigs, prostaglandin stimulation of the uterus is possible even in the presence of progesterone. Compounds with potent anti-fertile effects in guinea pigs tend to have poor activity in rats and vice versa. There also seems to be a reasonable correspondence of abortifacient potency in guinea pigs and that reported in humans. (Elger and Hasan, 1985 ACTA Physiologica Hungarica 65(4): 415-32).

In vitro Pharmacology Studies (see Appendix 1): The sponsor conducted in vitro studies to assess the potency of lubiprostone with other prostaglandin agonists: misoprostol (a PGE1 analogue), PGE1, PGE2 and PGF2α. The models used were guinea pig ileum longitudinal (EP1 receptor) and circular (EP2 receptor) smooth muscle, guinea pig vas deferens smooth muscle (EP3 receptor) and isolated iris sphincter muscle (FP receptor) from dogs. Compared to PGE1 and PGE2, lubiprostone and misoprostol had a very low affinity for EP1 and FP receptors. For the EP2 and EP3 receptors, lubiprostone had 20 to 40 fold lower affinity compared to misoprostol, PGE1 or PGE2. The affinity of PGE2 and PGF2α for the FP receptor was several magnitudes greater than for lubiprostone or misoprostol. Lubiprostone, misoprostol and PGF2α had weak EP1 agonist activity that did not produce maximal contraction of longitudinal smooth muscle, and lubiprostone was a substantially less potent agonist than misoprostol, PGE1 or PGE2 for EP2 receptors.

Although uterine tissues were not evaluated, these results suggest that lubiprostone would be much less potent in inducing uterine contractions than misoprostol and the natural prostaglandins.

Studies in Guinea Pigs (see Appendix 2):
Dose range finding study. Doses used in the definitive guinea pig study were based on the maximum tolerated dose identified in a dose range-finding study. Twelve presumed pregnant females/group were treated with 0, 5, 20, 40 or 80 µg/kg/day between on days 40 through 53. **Three females aborted prior to the initiation of treatment and were replaced with other animals.** Following initiation of treatment, mortalities were seen only in pregnant females at 5 (1/12), 40 (3/12) and 80 (3/12) µg/kg. Abortions were also seen at these doses: 2/9 at 5 µg/kg, 4/11 at 40 µg/kg, and 6/11 at 80 µg/kg. **At 20 µg/kg, no females died or aborted.** Based on these results, 25 µg/kg was chosen as the high dose for the definitive study.

Definitive Study. Groups in the definitive study were 0 (vehicle), 1, 10 and 25 µg/kg/day, an untreated control, and a PK satellite group. Main study groups consisted of two replicates for a total of 24 females/group, while the PK satellite groups had 5 pregnant females/group. In the main study groups, deaths in the vehicle and 1 µg/kg groups occurred after only 2-4 doses, while deaths and early sacrifices in the 25 µg/kg group occurred after 5-11 doses and were preceded by adverse clinical observations and decreases in body weight. The early deaths in the control and low-dose group are consistent with gavage errors, while the deaths in the high-dose group are suggestive that 25 µg/kg exceeded the maximum tolerated dose. The 2 abortions in the 10 µg/day animals occurred after 14 doses and in the 25 µg/kg group after 8-14 doses. *This long delay between initiation of dosing, combined with deaths occurring during the same time frame, suggests that maternal toxicity was a factor in the abortions.* Maternal toxicity at the higher dose is also supported by an increase in post-implantation lose in treated animals:

% Live fetuses at C-section as a function of number of implantations				
Untreated control	Vehicle control	1 µg/kg	10 µg/kg	25 µg/kg
100%	98%	96%	96%	90%

The sponsor has argued that the abortions observed in guinea pigs were related to stress and weight loss. *While guinea pigs are extremely sensitive to stress and have been shown to abort following dietary restriction, the data in this study suggest that the abortions were drug-related rather than due to stress.*

With regards to decreased weight gain and decreased food consumption, analysis of individual animal data demonstrated that there was no significant difference between animals that aborted and those that didn't. In addition, there were no difference in fetal or placental weights, or placental appearance.

In satellite animals, 3 females aborted 11, 2 and 4 days following the second blood draw, and the single death and 4th abortion occurred between the first and second blood draws. It should be noted that blood sampling is stressful on animals and data from these animals are therefore not generally included in the final study analysis for purposes other than pharmacokinetic monitoring.

Group	Pregnancies/group		Mortalities		Abortions	
	Main	Satellite	Main	Satellite	Main	Satellite
Untreated	21	3	0	0	0	0
0 (Vehicle)	17	5	1 (DG 43)*	0	0	0
1 µg/kg	21	5	2 (DG 41, 43)	1 (DG 52)	0	2 (DG 55, 57)
10 µg/kg	21	4	0	0	2 (DG 54, 58)	2 (DG 48, 54)
25 µg/kg	23	3	4 (DG 45, 47, 49, 50)	0	5 (DG 48, 49, 49, 51, 54)	0

*Day of Gestation in which deaths or abortions occurred.

Elger and Hasan found that appropriate doses of exogenous prostaglandins activate the quiescent guinea pig myometrium around gestation day 40 with a very short delay. They also found that the morphological integrity of the placenta deteriorated after treatment with prostaglandins, appearing hemorrhagic and the fetuses were usually dead hours before expulsion. Only PGE1 is an exception in this context since some pups have reportedly been born alive when treated during the final week of gestation.

Two different scenarios have been reported following exogenous prostaglandin administration to pregnant guinea pigs. On one hand, prostaglandins can induce prompt expulsion of the conceptuses without prior changes in serum progesterone while on the other, expulsions were exactly timed by a drop in serum progesterone below a critical level over a period of more than 10 days. In this study, abortions were not observed until after the 8th dose. Satellite animals which aborted had low progesterone levels; however, four other animals (2 controls, 1 at 1 µg/kg and 1 at 25 µg/kg) with low progesterone level were able to be carried to scheduled C-section indicative that even progesterone levels as low as 40 ng/ml were sufficient to maintain pregnancy in guinea pigs. (see table next page)

Progesterone Levels in Satellite Animals

GP #	Pregnancy Status	Progesterone levels (ng/ml)			Notes
		DG 39	DG 53	DG 60	
Untreated Control					
2374	NP	0.779	0.354	2.382	
7376	NP	4.812	1.170	6.442	
7075	P	142.300	128.100	95.103	
273	P	157.200	95.300	98.478	
5038	P	103.500	112.400	128.500	
Vehicle Control					
8600	P	**92.200**	**84.900**	**93.330**	
2330	P	145.700	113.900	139.300	
8797	P	147.300	106.200	133.400	
602	P	139.200	**82.400**	**87.521**	
9038	P	136.400	92.200	90.283	
1 µg/kg/day lubiprostone					
8305	P	30.888	-	-	Found Dead GD 52
582	P	146.00	**40.800**	**85.202**	
1304	P	128.400	124.900	101.948	
7876	P	36/472	15.885	-	Aborted GD 55 progesterone level 22.339 post-abortion
556	P	150.800	89.000	-	Aborted GD 57 progesterone level 65.967 post-abortion
10 µg/kg/day lubiprostone					
8516	NP	1.001	0.796	2.683	
9999	P	100.100	110.500	130.600	
8639	P	153.400	138.400	125.500	
9030	P	25.541	20.104	-	Aborted GD 55
376	P	40.887	-	-	Aborted GD 48 progesterone level 39.756 post-abortion
25 µg/kg/day lubiprostone					
6359	NP	1.591	0.751	0.628	
8046	NP	2.888	1.581	2.545	
7271	P	151.100	**66.675**	137.100	
3070	P	**42.861**	**62.270**	**84.384**	
2378	P	146.300	131.011	137.000	

Study in Monkeys (see Appendix 3): Based on body surface area, the doses used in the monkey study are approximately 6 and 20 times the proposed clinical dose of 24 µg/day. There were no adverse clinical observations, changes in body weight gain, food consumption, fetal or placental weights noted during treatment (DG 110-130) indicative that a maximum tolerated dose had been achieved. One animal (1/10) which had been dosed with 10 µg/kg/day aborted on DG 141, 11 days after cessation of treatment, and two females delivered normal/live fetuses on DG 149 (the day prior to scheduled C-sections): one in the 10 mg/kg group (1/9) and the other in the 30 mg/kg group (1/11). There was a large variability in progesterone levels with no evidence of a drug related effect. Historical control data in monkeys is reportedly 12.9% at the testing facility.

Table I-2 Incidences of reproductive findings in pregnant monkeys (after the initiation of dosing)

Dose (μg/kg)		0	10	30
No. of Pregnant Monkeys		10	10	11
Abortion/Early delivery	n	0	2	1
	(%)	0.0	20.0	9.1
Abortion	n	0	1	0
	(%)	0.0	10.0	0.0
Early delivery	n	0	1	1
	(%)	0.0	10.0	9.1

n : Number of pregnant monkeys
Not significantly different from control

Conclusion: While lubiprostone may have played a role in the abortions observed in guinea pigs and monkeys, the data is not conclusive. In guinea pigs the abortions could have been related to maternal toxicity, and the single abortion and early deliveries in monkeys are within historical control limits and could have been spontaneous. In vitro pharmacology data would indicate that when compared to natural prostaglandins and misoprostol, lubiprostone has only weak agonist activity in guinea pig ileum smooth muscle. The only definitive study would be a comparison of lubiprostone with a known abortifacient.

It is my recommendation that all reproductive data generated in the rat, rabbit, guinea pig be included in labeling.

c. Other drugs which cause fetal death but do not cause teratogenicity are generally labeled under Pregnancy Category C, and not recommended for use in pregnant women.

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APPENDIX 1

Effect of RU-0211 on Prostaglandin Receptors (Sponsor's Executive Summary 2.6.2)

RU-0211 is a unique PGE1 metabolite analogue. To assess potential prostaglandin activity, the effect of RU-0211 on three prostaglandin E receptors (EP1, EP2, and EP3) and one prostaglandin F receptor (FP) was investigated. The models used were guinea pig ileum longitudinal smooth muscle (EP1), guinea pig ileum circular smooth muscle (EP2), guinea pig vas deferens smooth muscle (EP3), and isolated iris sphincter muscle of the beagle (FP). In these assays, the effects of RU-0211 were compared with known prostaglandin agonists misoprostol (a PGE1 analogue), PGE1, PGE2, and PGF2α.

The smooth muscle preparations were suspended in organ baths containing 20 mL of Krebs-Ringer solution and maintained at a temperature of 32°C for longitudinal smooth muscle, and at 37°C for other smooth muscle preparations. The organ baths were aerated with 95%O2/5%CO2. Changes in muscle tension were recorded on an isometric transducer. Muscle preparations were exposed to increasing concentrations of agonist until a maximal response was achieved.

Responses were expressed as a percentage of the contraction induced by 10-5 M PGE2 for longitudinal smooth muscle (EP1) and 10-7 M PGF2α for contraction of iris smooth muscle (FP). Responses of circular smooth muscle of the ileum and smooth muscle of the vas deferens were recorded as inhibition of electrically induced twitch contractions. Responses were expressed as a percentage inhibition of the twitch response induced by 10-5 M PGE2 in circular smooth muscle (EP2) and 10-6 M PGE2 in the vas deferens (EP3). EC50 and IC50 values were calculated by probit analysis. Results are shown in Table 2.6.2.2-1.

Table 2.6.2.2-1. Comparative Effects of RU-0211 on EP and FP Receptors.

	Receptor Activity			
	EP_1 Ileum Longitudinal Smooth Muscle	EP_2 Ileum Circular Smooth Muscle	EP_3 Vas Deferens Smooth Muscle	FP Iris Circular Smooth Muscle
Compound	EC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	EC_{50} (nM)
RU-0211	54.1% at 10^{-5} M	581.4	49.6	28.4% at 10^{-5} M
Misoprostol	49.8% at 10^{-5} M	29.9	0.9	52.3% at 10^{-5} M
PGE_1	26.2	28.2	1.4	NT
PGE_2	47.0	52.2	1.9	403.4
$PGF_{2\alpha}$	44.6% at 10^{-5} M	6.6% at 10^{-5} M	258.2	4.5

NT: Not tested

RU-0211 had a very low affinity for EP1 receptors (Table 2.6.2.2-2; Figure 2.6.2.2-6). Maximum contraction of longitudinal smooth muscle (EP1) was not achieved at concentrations of 10-5 M. Misoprostol and PGF2α were also weak agonists that did not produce maximal contraction of longitudinal smooth muscle. Only PGE1 and PGE2 produced responses of sufficient magnitude to calculate EC50 values.

RU-0211 was a substantially less potent agonist on EP2 receptors than misoprostol, PGE1 or PGE2 (Table 2.6.2.2-3; Figure 2.6.2.2-7). Both misoprostol and PGE1 were approximately 20 times as potent and PGE2 was approximately 10 times as potent an agonist as RU-0211.

RU-0211 had low affinity for FP prostaglandin receptors (Table 2.6.2.2-5; Figure 2.6.2.2-9).

At a concentration of 10-5 M RU-0211 produced only 23% of the maximal response. The most potent agonist in this system was PGF2α.

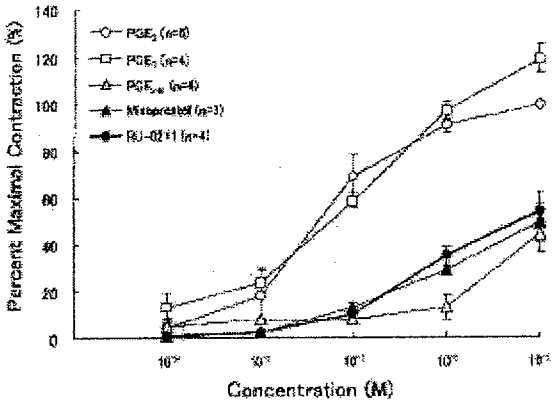
The results show that RU-0211 is a very weak agonist of all four prostaglandin receptors compared to the natural PGs and, therefore, is not expected to produce clinically significant pharmacologic effects mediated by these receptors.

Table 2.6.2.2-2. Effects of RU-0211, Misoprostol and Various PGs on Guinea Pig Ileum Longitudinal Muscle Preparations (EP_1 Receptor).

		Percent Maximal Concentration[1] (Mean ± SE, %)				
		Concentration (M)				
Compound	n	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
PGE_2	5	4.7 ± 2.8	18.6 ± 10.2	69.0 ± 9.2	91.1 ± 3.2	100
PGE_1	4	13.1 ± 6.5	23.9 ± 6.4	58.7 ± 3.2	97.6 ± 4.1	119.7 ± 5.7
$PGF_{2\alpha}$	4	5.1 ± 3.4	5.0 ± 0.9	6.4 ± 1.7	13.4 ± 5.2	44.6 ± 4.2
Misoprostol	3	1.2 ± 1.2	2.6 ± 0.7	12.5 ± 1.8	29.5 ± 1.2	49.8 ± 13.0
RU-0211	4	0.8 ± 0.5	2.8 ± 1.0	10.8 ± 3.6	36.2 ± 4.1	54.1 ± 3.4

1 The contraction after the addition of the test solution was expressed as a percentage of the contraction produced by PGE_2 at 10^{-5} M.

Figure 2.6.2.2-6. Dose response curve for the stimulation of contractions in the longitudinal muscle layer of isolated segments of guinea pig ileum (EP_1 receptor)



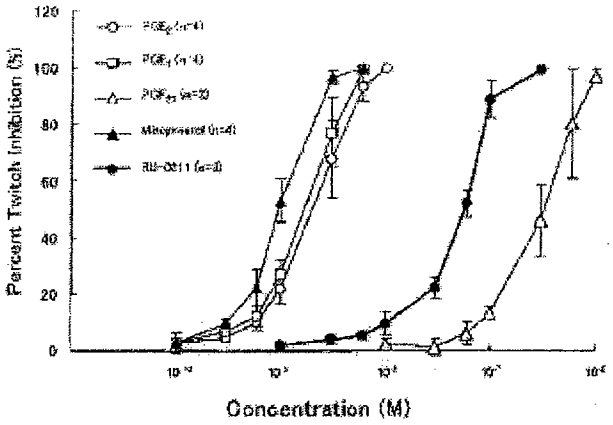
Data represent the mean ± SE of 3 to 5 determinations.

Table 2.6.2.2-3. Effects of RU-0211, Misoprostol and Various PGs on Electrically Stimulated Contraction of Guinea Pig Ileum Circular Muscle Preparations (EP_1 Receptor).

		Inhibition of Twitch Contraction[1] (Mean ± SE, %)				
		Concentration (M)				
Compound	n	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
PGE_2	5	4.5 ± 2.1	15.6 ± 3.0	58.0 ± 8.8	94.0 ± 4.0	100
PGE_1	3	-2.7 ± 4.3	14.2 ± 11.8	74.8 ± 24.1	101.0 ± 11.1	104.2 ± 3.6
$PGF_{2\alpha}$	3	-0.2 ± 2.4	1.8 ± 6.3	-0.2 ± 7.9	-1.3 ± 6.8	6.6 ± 2.9
Misoprostol	3	-0.5 ± 1.8	14.6 ± 5.5	68.0 ± 9.3	102.8 ± 3.2	106.5 ± 6.2
RU-0211	3	0.3 ± 1.2	4.8 ± 1.3	22.3 ± 4.9	47.5 ± 6.7	92.4 ± 4.2

1 The inhibition of electrically stimulated twitch contraction at each concentration of the test substance was expressed as a percentage of the inhibition induced with PGE_2 at 10^{-5} M.

Figure 2.6.2.2-8. Dose response curve for the inhibition of electrically stimulated twitch contraction in isolated segments of guinea pig vas deferens (EP_3 receptor)



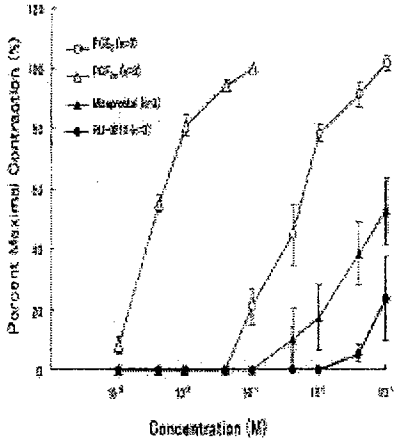
Data represent the mean ± SE of 3 to 4 determinations.

Table 2.6.2.2-5. Effects of RU-0211, Misoprostol and Various PGs on Contraction of Dog Iris Sphincter (FP Receptor).

		Percent Maximal Concentration[1] (Mean ± SE, %)								
		Concentration (M)								
Compound	n	10^{-9}	$4x10^{-9}$	10^{-8}	$4x10^{-8}$	10^{-7}	$4x10^{-7}$	10^{-6}	$4x10^{-6}$	10^{-5}
$PGF_{2\alpha}$	3	8.3 ± 3.0	54.9 ± 3.0	80.9 ± 3.5	94.3 ± 2.0	100	-	-	-	-
PGE_2	3	0	0	0	0	20.9 ± 6.0	44.6 ± 10.3	78.2 ± 2.9	91.2 ± 4.0	101.5 ± 2.5
Misoprostol	3	0	0	0	0	0	10.3 ± 10.3	17.1 ± 10.9	38.1 ± 10.9	52.3 ± 11.5
RU-0211	3	0	0	0	0	0	0	0	5.1 ± 2.9	23.4 ± 14.0

1 The contraction after the addition of the test solution was expressed as a percentage of the contraction produced by $PGF_{2\alpha}$ at 10^{-7} M.

Figure 2.6.2.2-9. Dose response curve for the stimulation of contractions in the iris sphincter segments of beagle dogs (FP receptor)



Data represent the mean ± SE of 3 determinations.

APPENDIX 2

Study SPI/SR05-009: Oral (Capsule) Abortion/Resorption Study of RU-0211 in Guinea Pigs (Sponsor's Executive Summary)

1. SUMMARY AND CONCLUSION

1.1. Purpose

The purpose of this study was to detect adverse effects, specifically abortion and/or resorption, following RU-0211 treatment of HA)BR timed-mated guinea pigs on days 40 through 53 of presumed gestation (DG).

1.2. Methods

One hundred-twenty timed mated female – HA)BR (Hartley) guinea pigs were randomly assigned to five dosage groups (Groups I through V), 24 guinea pigs per group. Two replicates (A and B) of 60 guinea pigs were assigned to the five dose groups, 12 guinea pigs per group per replicate. A replicate design was used to ensure sufficient resources at the Testing Facility to conduct the study and the limited number of timemated sows that could be supplied by the breeder. In addition, twenty-five additional guinea pigs (five per group) were randomly assigned to the study as satellite animals for blood sample collection to evaluate progesterone levels. Solutions of the test article, RU-0211, or the vehicle, medium chain fatty acid triglyceride, were administered orally via capsules once daily on DG 40 through 53 at doses of 0 (Untreated), 0 (Vehicle), 1, 10 and 25 mcg/kg/day. The dose volume was 0.5 mL/kg adjusted daily on the basis of the individual body weights recorded on the previous day.

The guinea pigs were checked for viability twice daily and examined for clinical observations and general appearance on the day of arrival at the Testing Facility and on DGs 15, 18, 21, 24, 27, 30, 33, 36 and 39 during the predosing period. The guinea pigs were also examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before dose administration and 60 ± 10 minutes after dose administration. These observations were recorded once daily during the postdosing period. Body weights were recorded on DG 0, the day of arrival at the Testing Facility, on DGs 15, 18, 21, 24, 27, 30, 33, 36 and 39, daily during the dosing and postdosing periods.

The criterion used to determine if a guinea pig had aborted was the delivery of one or more conceptuses or placentas. Several of the abortions were preceded by the presence of a red substance in the nesting box and/or a red perivaginal substance the day before or on the day of the incident. Aborted fetuses and concepti *in utero* were examined to the fullest extent possible using the same methods described for Caesarean-sectioned fetuses.

On DGs 39, 53 (before the last dose given) and 60, blood samples (approximately 1.5 mL each) were collected from the jugular artery of each guinea pig assigned to the satellite group. Blood samples were collected and processed for serum. The resulting serum was analyzed to determine progesterone levels.

All surviving guinea pigs were sacrificed by carbon dioxide asphyxiation on DG 60. Guinea pigs were Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of corpora lutea were recorded. The uterus of each guinea pig was excised and examined for pregnancy, number and distribution of implantation

sites, live and dead fetuses and early and late resorptions. Placentae were examined for size, shape and color.

1.3. Results

The following table summarizes the number of sows found dead, sacrificed because of moribund condition or that aborted and were sacrificed in the Untreated, 0 (Vehicle), 1, 10 and 25 mcg/kg/day dosage groups.

Text Table I. Summary of Mortality and Abortions[a] for Guinea Pigs Assigned to the Main (replicates A and B) and Satellite[b] Studies

Dosage Group	Study Assignment	Untreated	0 (Vehicle) mcg/kg/day	1 mcg/kg/day	10 mcg/kg/day	25 mcg/kg/day
Found Dead	Main	0	1[c]	2[d]	0	3[e]
	Satellite	0	0	1	0	0
Moribund Sacrificed	Main	0	0	0	0	1
	Satellite	0	0	0	0	0
Aborted/ Sacrificed	Main	0	0	0	2	5**##
	Satellite	0	0	2	2	0
Total		0	1[c]	5[d]	4	9[e]
Main		0	1[c]	2[d]	2	9[e]
Satellite		0	0	3	2	0

a. The criterion used to determine if a guinea pig had aborted was the delivery of one or more conceptuses or placentas. Several of the abortions were preceded by the presence of a red substance in the nesting box and/or a red perivaginal substance the day before or on the day of the incident. Aborted fetuses and concepti *in utero* were examined to the fullest extent possible using the same methods described for Caesarean-sectioned fetuses (See Section 2.8.6 for details).

b. Satellite - evaluated for progesterone levels.

c. Death occurred five minutes after administration of the fourth dose - death not related to test article.

d. One death occurred approximately 10 minutes after dosing - death not related to test article.

e. One death occurred during the dosing procedure - death not related to test article.

** Significantly different from the Group I value ($p \leq 0.01$).

Significantly different from the Group II value ($p \leq 0.01$).

The two abortions in the 10 mcg/kg/day dosage group occurred after 14 doses. These sows were gaining weight and had no adverse clinical observations. Deaths and/or moribund sacrifices in the 25 mcg/kg/day group occurred after 5 to 11 doses; abortions occurred in this group after 8 to 14 dosages. Both the deaths and abortions in the 25 mcg/kg/day dosage group were preceded by adverse clinical observations and reductions in body weight. Based on the above information, the abortions and deaths in the 0 (Vehicle), 1 and 10 mcg/kg/day dosage groups were presumed to be the result of trauma from the dosing or a spontaneous event (naturally occurring abortion) and not related to administration of RU-0211. Seven of the nine deaths, moribund sacrifices and abortions in the 25 mcg/kg/day dosage group were presumably related to RU-0211 administration because they occurred in the highest dose group in the presence of maternal toxicity.

The 25 mcg/kg/day dosages of RU-0211 was associated with adverse clinical signs including ungroomed coat, localized alopecia (particularly on the underside), cold to touch, red perivaginal substance and decreased motor activity (subjective observation, based on sow not attempting to move away or toward the observer). These clinical signs, which were observed in guinea pigs that were found dead or were sacrificed or aborted, occurred at a significantly increased incidences compared to the untreated and vehicle control group values. The observation of red substance in the nesting box or cage pan and/or red perivaginal substance was observed in three of five animals that aborted and was considered to be associated with or indicative of an impending abortion of the litter. The 10 mcg/kg/day dosage was not associated with any of the above adverse clinical signs.

Additional clinical observations noted in guinea pigs that were found dead, sacrificed or aborted in the 25 mcg/kg/day dosage group included tremors (subjective observation, shaking of the body while in place) red perioral substance, lacrimation, apparent dehydration (decreased skin turgor) scant feces, an abrasion on the mouth (likely due to dosing procedure), excess salivation, edema and gasping. Gasping was observed in one animal (3038) immediately following dosing indicating a problem in dosing.

The 1, 10 and 25 mcg/kg/day dosages of RU-0211 caused dosage-dependent reductions or significant reductions in body weight gains for the entire dosage and gestation periods. Body weight gains in the 0 (Vehicle), 1, 10 and 25 mcg/kg/day dosage groups were 100.0%, 78.9%, 78.9% and 47.3%, respectively, of the untreated control group values during the dosage period. During the postdosage period, body weight gains for sows in all RU-0211 dosage groups remained reduced by 11.1% to 33.3%. These effects of RU- 0211 on body weight gains resulted in significantly reduced body weights in the 25 mcg/kg/day dosage group beginning on DG 42 and continuing until scheduled sacrifice. There were no gross lesions considered related to RU-0211.

There were 17 to 23 pregnant guinea pigs in each dosage group. Reflecting the mortality and abortion pattern, observations from Caesarean-sections on DG 60 were based on 21, 16, 19, 19 and 14 pregnant sows in the five respective groups with one or more live fetuses, respectively. There were no significant differences between treatment groups and the untreated or treated control groups in litter size, number of live or dead fetuses, or numbers of resorptions..

Two fetuses in the 25 mcg/kg/day dosage group had multiple alterations noted at gross external examination; one of these two fetuses was dead. The findings were not statistically significant and therefore determined to be unrelated to the administration of RU-0211.

The progesterone data did not demonstrate any statistically significant differences between groups and there were no detectable trends probably due to the small number of pregnant sows evaluated and the significant variability within groups.

3.1. Mortality and Abortion
(Summaries - Tables 1 and 4; Individual Data - Tables 5, 11 and 13)

The following table (Text Table 1) summarizes the number of sows found dead, sacrificed because of moribund condition or those that aborted and were sacrificed in the Untreated, 0 (Vehicle), 1, 10 and 25 mcg/kg/day dosage groups.

Text Table 1. Summary of Mortality and Abortions[a] for Guinea Pigs Assigned to the Main (replicates A and B) and Satellite[b] Studies

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	Satellite	0	0	1	0	0
Moribund Sacrificed	Main	0	0	0	0	1
	Satellite	0	0	0	0	0
Aborted/ Sacrificed	Main	0	0	0	2	5**##
	Satellite	0	0	2	2	0
Total		**0**	**1**[c]	**5**[d]	**4**	**9**[e]
Main		0	1[c]	2[d]	2	9[e]
Satellite		0	0	3	2	0

a. The criterion used to determine if a guinea pig had aborted was the delivery of one or more conceptuses or placentas. Several of the abortions were preceded by the presence of a red substance in the nesting box and/or a red perivaginal substance the day before or on the day of the incident. Aborted fetuses and concepti *in utero* were examined to the fullest extent possible using the same methods described for Caesarean-sectioned fetuses (See Section 2.8.6 for details).

b. Satellite - evaluated for progesterone levels.

c. Death occurred five minutes after administration of the fourth dose - death not related to test article.

d. One death occurred approximately 10 minutes after dosing - death not related to test article.

e. One death occurred during the dosing procedure - death not related to test article.

** Significantly different from the Group I value ($p \leq 0.01$).

Significantly different from the Group II value ($p \leq 0.01$).

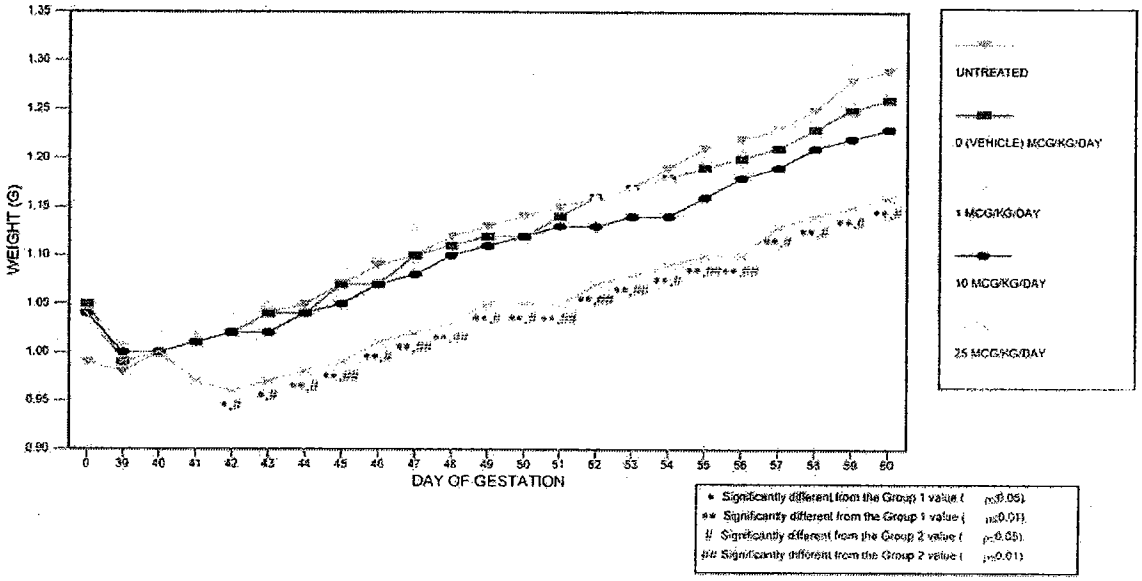
Deaths in the 0 (Vehicle) and 1 mcg/kg/day dosage groups occurred after 2 to 4 doses. The two abortions in the 10 mcg/kg/day dosage group occurred after 14 doses. These sows were gaining weight and had no adverse clinical observations. Deaths and/or moribund sacrifices in the 25 mcg/kg/day group occurred after 5 to 11 doses; abortions occurred in this group after 8 to 14 dosages. Both the deaths and abortions in the 25 mcg/kg/day dosage group were preceded by adverse clinical observations and reductions in body weight. These clinical observations included ungroomed coat, localized alopecia (particularly on the underside), cold to touch, red perivaginal substance, decreased motor activity, tremors, red perioral substance, lacrimation,

PROTOCOL 4619-002: ORAL (CAPSULE) ABORTION/RESORPTION STUDY OF RU-0211 IN GUINEA PIGS

MATERNAL BODY WEIGHTS

REPLICATES A AND B COMBINED

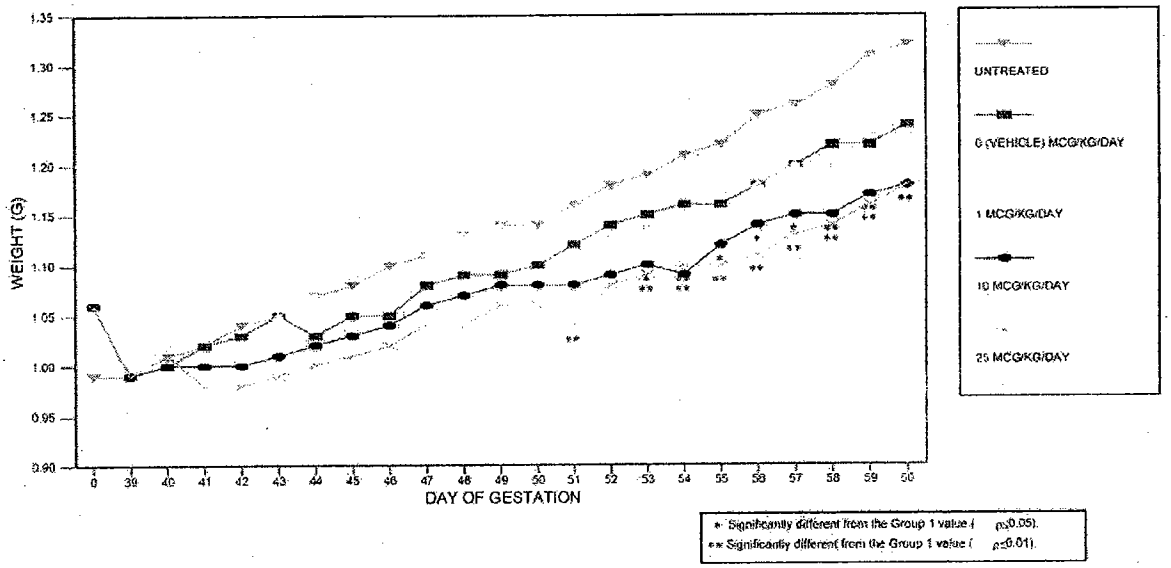
Figure 1



PROTOCOL 4619-002: ORAL (CAPSULE) ABORTION/RESORPTION STUDY OF RU-0211 IN GUINEA PIGS

MATERNAL BODY WEIGHTS
REPLICATE A

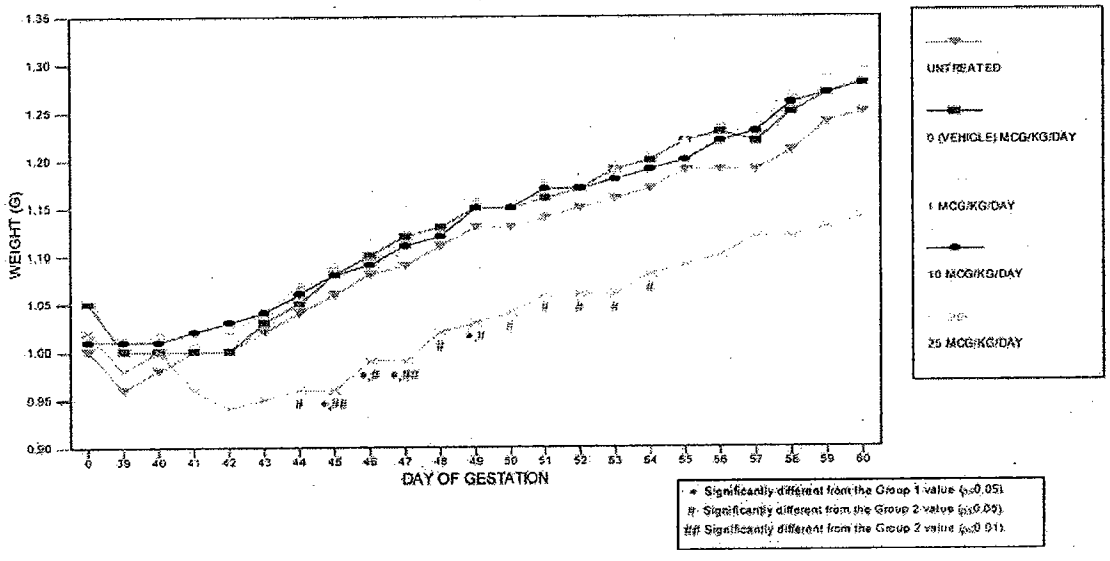
Figure 2



PROTOCOL 4619-002: ORAL (CAPSULE) ABORTION/RESORPTION STUDY OF RU-0211 IN GUINEA PIGS

MATERNAL BODY WEIGHTS
REPLICATE B

Figure 3



APPENDEX 3

Study SPI/SR05-001: A Study to Evaluate the Abortifacient Effect of RU-0211 in Rhesus Monkeys by Oral Gavage Administration (Sponsor's Executive Summary)

Study Design

Pregnant animals were assigned to dose groups including vehicle control group. Dose levels were 0 (vehicle control), 10, and 30 µg/kg/day (Table 1).

Table 1 Study Groups

Group/ Color Card	Test or Control Article	Dose Level (µg/kg)	Dose Conc. (µg/mL)	Dose Volume (mL/kg)	Number of Pregnant Animals (Animal No.)
1 / blue	Vehicle	0	0	0.1	10 (10001 to 10010)
2 / green	RU-0211	10	100	0.1	10 (10101 to 10110)
3 / yellow	RU-0211	30	300	0.1	11 (10201 to 10211)

Justification for Selection of the Dose Levels

The dose levels selected for the current study were based upon the previously completed reproduction studies in rats and the published literature. In the rat studies, the NOEL was determined to be 0.2 mg test article/kg body weight/day. In addition, published data indicates that rhesus monkeys are 3 to 20 times more sensitive than rats to the abortifacient effects of a class of compounds that are similar to the test article [3]. Therefore, it was believed that the doses selected for the current study in rhesus monkeys (10 and 30 µg/kg/day) allowed for the abortifacient effect of test article to be determined without overt maternal toxicity. In addition, these doses were approximately 4X and 12X the proposed clinical doses (based upon mg/m^2).

RESULTS

A. Pregnant Non-Human Primates

1. Clinical Observations (Tables 1-1 and 1-2)

No females died, and no abnormalities were observed in clinical signs in any group. Early delivery on Day 149 of gestation was observed in 1 animal (No. 10205) in the 30 µg/kg group and in 1 animal (No. 10107) in the 10 µg/kg group. An abortion on Day 141 of gestation was observed in 1 animal (No. 10104) in the 10 µg/kg group. However, no significant differences were noted in these incidences between the control and test article groups.

2. Food Consumption (Table 2)

No significant differences were noted in food consumption between the control and test article groups.

3. Body Weight (Table 3)

No significant differences were noted in body weight between the control and test article groups.

4. Serum Progesterone Concentrations (Table 4)

No significant differences were noted in serum progesterone concentrations between the control and test article groups.

B. Premature Delivery

Early delivery on Day 149 of gestation was observed in 1 animal (No. 10205) in the 30 μg/kg group and in 1 animal (No. 10107) in the 10 μg/kg group. These neonates were delivered naturally and alive. No lactational abnormalities were observed in these monkeys. No abnormalities were observed in external features or general health condition in the neonates, which were female and male in the 30 and 10 μg/kg groups, respectively. The body weights of these neonates were 337g and 413g in the 30 and 10 μg/kg groups, respectively.

Table 1-2 Incidences of reproductive findings in pregnant monkeys (after the initiation of dosing)

Dose (μg/kg)		0	10	30
No. of Pregnant Monkeys		10	10	11
Abortion/Early delivery	n	0	2	1
	(%)	0.0	20.0	9.1
Abortion	n	0	1	0
	(%)	0.0	10.0	0.0
Early delivery	n	0	1	1
	(%)	0.0	10.0	9.1

n : Number of pregnant monkeys
Not significantly different from control

C. Fetuses

1. External Examination, Body and Placental Weights (Tables 5-1 and 5-2)

No external abnormalities were observed in any fetus. No significant differences were noted in fetal body weight or placental weight between the control and test article groups. Fused placenta and single placenta were observed in the 10 μg/kg group, however, no significant differences were noted in their incidences when compared with the control group.

DISCUSSION

RU-0211 was administered by oral gavage (nasogastric) to 10 or 11 pregnant rhesus monkeys in each group at dose levels of 0 [medium chain fatty acid triglyceride (MCT)], 10, and 30 μg/kg/day during the late gestation period (from Days 110 to 130 of gestation, total 21 days), in order to evaluate the abortifacient effects on non-human primates.

No monkeys died, and no abnormalities were observed in clinical signs, food consumption, body weight, or serum progesterone concentrations in any group. Early delivery on Day 149 of gestation was observed in 1 animal in the 30 μg/kg group and in 1 animal in the 10 μg/kg group. The neonates were delivered naturally and alive. No lactational abnormalities were observed in these monkeys. No abnormalities were observed in body

weight, external features or general health condition in these 2 neonates. Historical control data in SNBL DSR[61] has shown natural deliveries between GD 144 to 171. Therefore, these natural deliveries on GD 149 were within the historical control range and considered normal. An abortion on Day 141 of gestation was observed in 1 monkey in the 10 µg/kg group. However, this was considered incidental, because the incidence of this premature delivery (before day 150 of gestation) was within the range of the historical control data (mean: 12.9%)[61] and no abortions occurred in the 30 µg/kg group.

No test article-related changes were noted in fetal external examinations, fetal body weights, placental examinations, or placental weights in the 30 or 10 µg/kg groups.

Under the conditions of this study, it was concluded that RU-0211 was not an abortifacient.

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Ronald Orleans
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Scott Monroe
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MEDICAL OFFICER
I concur with the recommendations of Dr. Orleans.

Daniel A. Shames
12/23/2005 03:31:09 PM
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