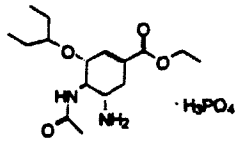
CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER for: 021087

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS:	
REVIEWER NAME:	Ita Yuen
DIVISION NAME:	Division of Antiviral Drug Products
HFD#:	530
REVIEW COMPLETION DATE:	10/15/99
ELECTRONIC FILE NUMBER:	None
NDA NUMBER:	21,087
SERIAL #/DATE/TYPE OF SUBMISSION:	000/April 29,1999/Original
INFORMATION TO SPONSOR:	Yes ( ) No (X)
SPONSOR (OR AGENT):	Hoffmann-La Roche Inc. 340 Kingsland Street Nutley, NJ 07110-1199
MANUFACTURER OF DRUG SUBSTANCE:	F. Hoffmann-La Roche Ltd. Grenzacherstrasse 124 CH-4070 Basel, Switzerland
DRUG:	[redacted]
Generic Name:	Oseltamivir phosphate
Trade Name:	Tamiflu®
Chemical Name:	(3R,4R,5S)-4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate (1:1)
CAS Registry Number:	204255-11-8
Molecular Formula/Molecular Weight:	$C_{16}H_{28}N_2O_4$ (free base)/M.W. = 312.41 $C_{16}H_{28}N_2O_4$ 1:1 H_3PO_4 (phosphate salt)/410.408
Structure:	
RELEVANT INDS/NDAS/DMFS:	IND [redacted] DMF [redacted] DMF [redacted] DMF [redacted]
DRUG CLASS:	Influenza viral neuraminidase inhibitor
INDICATION:	Treatment of influenza
CLINICAL FORMULATION:	The drug product is being supplied as 75-mg [redacted] The excipients contain [redacted] pre-

gelatinized starch [redacted] Povidone K 30 [redacted]
croscarmellose sodium [redacted] Talc [redacted]
sodium stearyl fumarate.

ROUTE OF ADMINISTRATION: Oral

PROPOSED CLINICAL USE: Treatment of influenza

DISCLAIMER: Some material may be taken directly from sponsor's submission

INTRODUCTION AND DRUG HISTORY:

Ro 64-0796 is an oral ethyl ester prodrug of an anti-influenza agent Ro 64-0802, which has poor bioavailability via the oral route of administration. Ro 64-0802 binds specifically to the active site of the neuraminidase enzyme on the surface of the influenza virus. This enzyme is one of the 2 major surface antigens present on the virion surface of both influenza virus types A and B, the viral types most frequently associated with morbidity in humans. Four antigenically distinct influenza virus types have been cultured since 1933. They are distinguished by the surface antigens, the hemagglutinin (H) and neuraminidase (N). There are 15 subtypes of hemagglutinin and 9 antigenically distinct neuraminidase enzymes identified. However, the active site on the different neuraminidase subtypes is highly conserved. This is the rationale for designing a neuraminidase inhibitor for treatment and prevention of influenza viral infection.

The pandemic influenza infection in 1918-1919, the "Spanish flu," was responsible for 20 million deaths worldwide. Center for Disease Control has begun to develop a pandemic plan to limit morbidity, mortality, and social disruption associated with pandemic outbreaks of influenza viral infection. In addition to vaccine, there are a total of 3 agents approved for the treatment of influenza infection, namely amantidine, rimantidine, and zanamivir. The 1st 2 drugs inhibit the activity of the M2 protein of influenza A virus but are inactive against influenza B virus. Zanamivir is another influenza neuraminidase inhibitor like Ro 64-0802. However, it is administered by inhalation. The sponsor has argued for the need of additional anti-influenza agents that will be effective against both type of influenza viruses to be available as soon as possible and requested for a priority review of the present NDA. The request was granted.

The sponsor has pursued an aggressive clinical and nonclinical drug development. The original IND was submitted on April of 1997 for a clinical trial to be conducted during the Southern Hemisphere's flu season that summer. The original submission contained nonclinical toxicology studies to support the proposed 5-day treatment plan for influenza viral infection and the 2-week seasonal prophylaxis program to prevent influenza viral infection. Because of the short duration of the treatment and prophylaxis programs, the sponsor requested for a waiver of the required carcinogenicity studies. However, since the drug will be given possibly annually to both children and adults who are otherwise healthy to prevent a disease that is generally non-life threatening, it was felt that chronic toxicity and carcinogenicity need to be studied. The waiver was not granted. Thus, the sponsor was advised not to include a prophylaxis claim in the present NDA without submitting the results of the 2-year carcinogenicity studies for review.

The present NDA submission contains all of the required general toxicology, reproductive toxicology, and genotoxicity studies to support the clinical trials and Label claims.

STUDIES REVIEWED WITHIN THIS SUBMISSION:

Studies and their reviews are listed and found under the relevant sections.

STUDIES NOT REVIEWED WITHIN THIS SUBMISSION:

[redacted]

APPEARS THIS WAY
ON ORIGINAL

TOXICOLOGY

Study Summary:

A. Single Dose Toxicology Studies:

A1. Ro 64-0796/002 (GS 4104): An acute intravenous toxicity study in the mouse (Report # W-142710; Study # AM672; Roche Products Ltd., Welwyn Garden City, England; Lot # 4104-02-B-1; GLP; With QA-report; Study dates 1/29/97-2/19/97; Vol. 19, pp. 1-35).

A2. Ro 64-0796/002: Acute oral (intubation) toxicity study in mice (Report # N-181235; Study No. 07113; Hoffmann-La Roche Inc., Nutley, NJ; Lot # 71121003; GLP; With QA-report; Study dates 7/30/98-8/13/98; Vol. 19, pp. 36-74).

A3. Ro 64-0796/002: Acute oral (intubation) toxicity study in rats (Report # N-181236; Study # 07114; Hoffmann-La Roche Inc., Nutley, NJ; Lot # 71121003; GLP; With QA-report; Study dates 7/30/98-8/13/98; Vol. 19, pp. 75-111).

A4. Ro 64-0796 (also known as GS4104): Oral (gavage) acute toxicity study in juvenile rats (Report # W-143027; Study # 276/99-D6154; [redacted] Lot # 80202143; GLP; With QA-report; Study dates 8/21/98-9/4/98; Vol. 19, pp. 112-155).

A5. Ro 64-0792/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167927; Study # 018I98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 71201840; GLP; With QA report; Study dates 2/10/98-2/24/98; Vol. 57, pp. 1-19).

A6. Ro 64-0789/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167926; Study # 017I98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 7100240; GLP; With QA report; Study dates 2/10/98-2/24/98; Vol. 57, pp. 20-38).

A7. Ro 64-0795/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167928; Study # 019I98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 7110052161; GLP; With QA report; Study dates 2/10/98-2/24/98; Vol. 57, pp. 39-58).

B. Repeat Dose (Subchronic & Chronic) Studies

B1. Ro 64-0796 (also known as GS4104): Five day repeated dose oral toxicity study of [redacted] 4104 in male Sprague-Dawley rats (Report # RR W-142693; Study # 96-TOX-4104-001; [redacted] Lot # 1031-47; non-GLP; Without QA report; Study dates 2/12/96-2/19/96; Vol. 22, pp.1-46).

B2. Ro 64-0796 (also known as GS 4104): Fourteen day repeated dose oral toxicity study of [redacted] 4104 in Sprague-Dawley rats (Report # W-142694; Study # 96-TOX-4104-002; [redacted] Lot # 1029-49; non-GLP; Without QA report; Study dates 4/2/96-4/16/96; Vol. 22, pp. 47-151).

B3. Ro 64-0796 (also known as [redacted] 4104): A 14-day gavage toxicity study of GS-4104 in the albino rat (Report # W-142696; Study # 96-TOX-4104-004; [redacted] Lot # 1132-3-14; GLP, With QA report; Study dates 8/27/96-10/2/96; Vols. 23-24).

B4. Ro 64-0796/002 ([redacted] 4104): A one month oral (gavage) toxicity and toxicokinetic study in the rat (Report # W-142761; Study # SAR677; Roche Discovery Welwyn, Herts, UK

Lot # 4104-02-B-4; GLP; With QA report; Study dates 5/8/97-6/19/97; Vols. 25-26).

B5. Ro 64-0796/002 (also known as GS4104): A 4-week oral (dietary administration) range-finding and palatability toxicity study in the rat (Report # W-142979; Study # 276/90-D6154; Lot # 80202143; GLP; With QA-report; Study dates 4/6/98-5/5/98; Vol. 27).

B6. Ro 64-0796/002: A 27-week oral (intubation) toxicity study in rats followed by an 8-week recovery period, and a 27-week oral (intubation) plasma concentration study in rats (Report # N-138531 (doses of 50, 200, & 1000 mg/kg/day) & N-138539 (dose of 100 mg/kg/day); Study #'s 06992 & 06994 (doses of 50, 200, & 1000 mg/kg/day) & 07011 and 07012 (dose of 100 mg/kg/day); Hoffmann-La Roche, Nutley, NJ (toxicity study) Lot #'s 4104-02-F-2, GPM0176, 71121001, 71121002, & 71121003; GLP; With QA-report; Study dates 8/25/97-8/31/98; Vols. 28-32 for doses of 50, 200, & 1000 mg/kg/day & Vols. 33-35 for the dose of 100 mg/kg/day).

B7. Ro 64-0796 (also known as 4104): Expert assessment of the kidneys from the rat toxicity studies. (Report # W-143032; Study #'s 87900, SAR 677, 06992/07011 Study dates 11/29/97-9/16/98; Vol. 36, pp. 1-18).

B8 Ro 64-0796 (also known as 4104): A 14-day range-finding oral toxicity study in juvenile rats (Report # W-143030; Study 71-53; Lot # 80202143; GLP; With QA-report; Study dates 5/25/98-10/20/98; Vol. 36, 19-165).

B9: Ro 64-0796 (also known as 4104): A two-week oral toxicity study in juvenile rats aged 7-20 days (Report # W-143066; Study 71-54; Lot #'s 80202143; GLP; With QA-report; Study dates 5/25/98-1/25/99; Vol. 37).

B10. Ro-0796 (also known as 4104): A four-week oral toxicity study in juvenile rats aged 21-49 days (Report # W-143067; Study 71-55; Lot # 80202143; GLP; With QA-report; Study dates 8/10/98-1/29/99; Vol. 38).

B11. Ro 64-0802/002: A 2 week intravenous toxicity and toxicokinetic study in the rat (Report # W-142938; Study # SAR691; Roche Products Ltd., Welwyn Garden, Herts, UK; Lot # 4071-01-A-1; GLP; With QA report; Study dates 2/19/98-3/5/99; Vol. 39).

B12. Ro 64-0796/001: A preliminary 7 day oral toxicity and toxicokinetic study in the marmoset (Report # W-142646; Study # SAP668E; Roche Products Ltd., Welwyn Garden, Herts, UK; Lot # 1029-92; GLP; With QA report; Study dates 7/23/96-8/2/96; Vol. 40).

B13. Ro 64-0796 (also known as 4104): 28-day oral (gavage) study in the marmoset monkey with a 14 day treatment-free period (Report # RR W-142697; Study # 96-TOX-4104-003; Lot # 1132-5-14; GLP; With QA-report; Study dates 9/3/96-10/15/96; Vols. 41-42).

B14. Ro 64-0796/002: A 9 month oral toxicity and toxicokinetic study in the marmoset (Report # W-143011; Study # SCP681; Roche Products Ltd., Welwyn Garden, Herts, UK (toxicity study) Lot # 4104-02-F2; GLP; With QA-report; Study dates 8/7/97-7/8/98; Vol. 43).

B15. Ro 64-0802/002: A 14 day intravenous repeated dose toxicity study of Ro 64-0802/002 in male marmosets (Report # W-142940; Study 71-51;

[redacted] Lot # 4071-01-A-1; GLP; With QA-report; Study dates 2/10/98-5/25/98; Vol. 44, pp. 1-77).

Study Review:

A1. Ro 64-0796/002[redacted]4104): An acute intravenous toxicity study in the mouse (Report # W-142710; Study No. AM672; Lot # 4104-02-B-1). A single dose of 5, 50, or 250 mg/kg Ro 64-0796/002 was administered to 1 CD-1 mouse/sex/dose via the tail vein. The male dosed at 250 mg/kg convulsed and died almost immediately after dosing. The female dosed at the same level also convulsed for 15 minutes after dosing, had panting respiration, unsteady gait, and intermittent body tremors, but survived to scheduled necropsy. Another group of 1 mouse/sex was treated with 100 mg/kg via intravenous administration. Except for slight piloerection observed in one male, no clinical signs were noted in all the other treatment groups. A fifth group of 5 mice/sex received a single dose of 100 mg/kg and observed for 2 weeks. Except for the blackening and/or scabbing in 2 females, no other treatment-related effects were noted. Thus, the Maximum Non-Lethal Dose Level (MNLD), following intravenous administration to the mouse, was considered to be between 100 and 250 mg/kg.

A2. Ro 64-0796/002: Acute oral (intubation) toxicity study in mice (Report # N-181235; Study No. 07113; Lot # 71121003). Five CD-1 mice/sex/group were administered a single dose of vehicle (acetate buffer, pH 4.0) or 2000 mg/kg Ro 64-0796/002 by oral gavage and observed for 14 days. No mortality, clinical signs, or macroscopic changes were observed. The treated females failed to gain weight between days 7 and 14. The MNLD, following a single oral administration, is considered to be greater than 2000 mg/kg in mice.

A3. Ro 64-0796/002: Acute oral (intubation) toxicity study in rats (Report # N-181236; Study # 07114; Lot # 71121003). Five Sprague-Dawley rats/sex/group were administered a single dose of vehicle (acetate buffer, pH 4.0) or 2000 mg/kg Ro 64-0796/002 by oral gavage and observed for 14 days. No treatment-related effects were observed. The MNLD, following a single oral administration, is considered to be greater than 2000 mg/kg in rats.

A4. Ro 64-0796 (also known as [redacted]4104): Oral (gavage) acute toxicity study in juvenile rats (Report # W-143027; Study # 276/99-D6154; Lot # 80202143). Six litters of 14 days old (not weaned until 21 days *post-partum*) Crl:CD.BR pups culled randomly to 5/sex received a single dose of vehicle (acetate buffer, pH 4.0), 250, 500, 1000, 1500, or 2000 mg/kg Ro 64-0796/002 via oral gavage and were observed for 14 days. One female in the 2000 mg/kg group was sacrificed in moribund condition due to dosing error. No treatment-related effects were seen.

A5. Ro 64-0792/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167927; Study # 018198; Lot # 71201840). Ro 64-0792/000 is an epoxide, the starting material for the manufacturing of Ro 64-0796. It can be prepared from (-)-quinic acid, (-)-shikimic acid, or D(+)-mannose. Ro 64-0792 is opened by nucleophilic attack of sodium azide in the presence of ammonium chloride to yield predominantly the 5-azido alcohol, Ro 64-0793. The acute toxicity of this intermediate was tested in 5 Wistar rats/sex at a single oral dose of 2000 mg/kg. The rats were observed for 14 days and necropsy performed. Hydronephrosis was seen in both kidneys of one female. The corresponding microscopic finding

was bilaterally single inflammatory cell foci in the outer medulla. Similar inflammatory cell foci were found in one or both kidneys of 5 out of 10 rats. Thus, the maximum tolerated dose for this study was 2000 mg/kg.

Comment: The necropsy and histopathology reports were not included in the report. Thus, it's unclear which 5 animals (i.e., how many males and females) had the inflammatory cell foci in the kidneys.

A6. Ro 64-0789/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167926; Study # 017198; Lot # 7100240). Ro 64-0789/000 is the first common intermediate in the synthesis of Ro 64-0792 (the starting material for the synthesis of Ro 64-0796) from (-)-quinic acid, (-)-shikimic acid, or D(+)-mannose. The acute toxicity for this intermediate was tested in 5 Wistar rats/sex administered at a single dose of 2000 mg/kg. The rats were observed for 14 days and necropsy performed. No treatment effects were observed. Thus, the maximum tolerated dose for this intermediate was greater than 2000 mg/kg.

Comment: The necropsy and histopathology reports were not included in the report.

A7. Ro 64-0795/000: Acute toxicity study in the rat by oral administration (gavage) fixed dose method (Report # B-167928; Study # 019198; Lot # 7110052161). Ro 64-0795/000 is the direct synthetic precursor of the neuraminidase inhibitor (Ro 64-0796). It is an azido-acetamide and contains a specified impurity Ro 64-2988, a bis-azide. Partial reduction of this impurities gives rise to a 2-azido impurity, Ro 64-1637, which was tested positive in *Salmonella typhimurium* strains TA1535. The acute toxicity for this intermediate was tested in 5 Wistar rats/sex administered at a single dose of 2000 mg/kg. The rats were observed for 14 days and necropsy performed. No treatment effects were observed. Thus, the maximum tolerated dose for this intermediate was greater than 2000 mg/kg.

Comment: The necropsy and histopathology reports were not included in the report.

B1. Ro 64-0796 (also known as [redacted]4104): Five day repeated dose oral toxicity study of GS-4104 in male Sprague-Dawley rats (Report # RR W-142693; Study # 96-TOX-4104-001; Lot # 1031-47). Five male Sprague Dawley rats/group were administered sterile water or 300 mg/kg/day Ro 64-0796 at dosing volume of 10 mg/kg/day for 5 consecutive days. Clinical observation (daily), body weight measurement (study days 1 and 5), gross necropsy examination, clinical chemistry, hematology, and histopathological analysis (at termination, study day 8) were performed. Organs evaluated microscopically included lung, liver, kidneys, adrenals, spleen, testes, thymus, heart, bone marrow, gastrointestinal tract, and all gross lesions. There were no treatment effects on all the endpoints analyzed. Thus, the no-observable-effect level (NOEL) exceeds 300 mg/kg/day.

B2. Ro 64-0796 (also known as [redacted]4104): Fourteen day repeated dose oral toxicity study of [redacted]4104 in Sprague-Dawley rats (Report # W-142694; Study # 96-TOX-4104-002; Lot # 1029-49). Four Sprague-Dawley rat/sex/dose were administered sterile water, 40, 160, or 800 mg/kg/day Ro 64-0796 at a dosing volume of 10 mg/kg/day for 14 days. Clinical observation (daily), body weight measurement (study days 1, 5, and 10), gross necropsy examination, clinical chemistry, hematology, and histopathological analysis (at termination, study day 15) were

performed. Organs evaluated microscopically included lung, liver, kidneys, adrenals, spleen, brain, gonads, thymus, thyroid, urinary bladder, heart, bone marrow, pancreas, gastrointestinal tract, and all gross lesions. There was a very slight, but statistically significant increase in relative liver weight and ALT level in the high dose females. No other effects were attributable to the treatment. Thus, the Low-observable-effect level (LOEL) is 800 mg/kg/day.

B3. Ro 64-0796 (also known as 4104): A 14-day gavage toxicity study of GS-4104 in the albino rat (Report # W-142696; Study # 96-TOX-4104-004; Lot # 1132-3-14).

Species/Strain: Sprague-Dawley rats	Route: Oral (gavage)	Vehicle: Sterile water	Dose Volume: 10 ml/kg
Weight Range: M = 184-233 g; F = 143-182 g	Age on Day 1: 7 weeks old		Duration of Dosing: 14 days

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Daily	Gross pathology	Termination
Body weight	Daily	Organ weights	Termination
Food consumption	Weekly	Histopathology	Termination on control & high dose only
Hematology	Day 15	Toxicokinetics	Days 1 and 14 at predose, 0.5, 1, 2, 4, 8, 12,
Clinical chemistry	Day 15		and 24 hrs postdose

Important findings

Clinical observations	Salivation was seen in all animals dosed with 2000 mg/kg/day and most of the animals receiving 500 mg/kg/day throughout the treatment period. Red and/or yellow fur staining in the urogenital region was seen in 4/18 males and 7/18 females on days 8-9 and in 14/18 males and 15/18 females on day 15 in the high dose (2000 mg/kg/day) groups.
Body weights	There was a dose-related reduction in the body weights in the treated males. Although they only reached statistical significance (8-10% lower than the concurrent controls from days 3-14) in the high dose group (2000 mg/kg/day). Body weights were similar in the females.

Sex		**Males**				**Females**			
Daily Dosage (mg/kg/day)		0	125	500	2000	0	125	500	2000
Number of animals:									
Main study		10	10	10	10	10	10	10	10
Toxicokinetic[#]		0	8	8	8	0	0	0	8
$AUC_{0\rightarrow 24h}$ (µg-h/ml)	**Day 1**	-	25.2	135	642	-	-	-	398
	Day 14	-	37.2	168	843	-	-	-	615
C_{max} (µg/ml)	**Day 1**	-	5.03	21.9	55.9	-	-	-	23.9
	Day 14	-	6.15	21.7	74.5	-	-	-	50.7
C_{min} (µg/ml)	**Day 1**	-	0.00	0.44	9.86	-	-	-	8.26
	Day 14	-	0.00	0.22	10.7	-	-	-	7.45
T_{max} (h)	**Day 1**	-	1.0	2.0	8.0	-	-	-	8.0
	Day 14	-	1.0	2.0	8.0	-	-	-	8.0
Number of Deaths:									
Cause: Accident		0	0	1	1	0	0	0	0
Pulmonary congestion		0	0	0	0	0	0	0	1
Hematology:									
Erythrocytes (X 10^6)		6.84	6.93	6.81	7.07	6.78	6.95	6.77	7.36**
Hemoglobin (g/dl)		14.5	14.7	14.5	15.0	14.8	14.9	14.7	15.7**
Hematocrit (%)		41.4	42.3	41.5	42.6	40.6	41.2	40.4	43.2**
Red cell distr. width (%)		12.7	12.6	12.9	13.6	12.6	12.6	12.8	13.4**

Sex	Males				Females			
Daily Dosage (mg/kg/day)	0	125	500	2000	0	125	500	2000
Number of animals:	10	10	10	10	10	10	10	10
Relative organ weights (%):								
Heart	0.403	0.421	0.429	0.353*	0.431	0.440	0.478*	0.423
Adrenals	0.019	0.021	0.020	0.021	0.032	0.033	0.033	0.040**
Kidneys	0.880	0.885	0.889	0.966*	0.905	0.895	0.895	1.011**
Gonads	0.993	1.026	1.083	1.112**	0.048	0.047	0.047	0.049
Thymus	0.252	0.234	0.254	0.198*	0.258	0.298	0.277	0.230
Clinical chemistry:								
Urea nitrogen (mg/dl)	12.2	12.2	13.8	18.1**	14.1	15.5	14.6	19.2**
Creatinine (mg/dl)	0.5	0.6	0.6	0.5	0.5	0.6	0.6	0.6**
Glucose (mg/dl)	132.8	136.1	137.0	170.9**	136.7	142.3	128.9	161.1
Calcium (mg/dl)	9.9	10.1	10.2	11.0**	9.8	10.2	10.2	11.3**
Phosphorus (mg/dl)	9.87	9.96	10.17	10.65*	8.96	9.47	9.45	10.69**
Cholesterol (mg/dl)	61.9	60.5	57.5	85.4**	72.4	79.0	80.4	88.3
Total protein (g/dl)	5.8	5.8	5.8	6.0**	5.9	5.9	6.0	6.4**
Globulin (g/dl)	2.7	2.8	2.8	2.9*	2.7	2.7	2.8	3.0**
Sodium (meq/l)	143.4	144.3	143.9	141.8**	143.8	142.8	144.3	140.1**
Potassium (meq/l)	4.77	4.59	4.63	4.24**	4.35	4.51	4.24	4.00*
Chloride (meq/l)	100.3	100.7	98.6	94.1**	100.4	100.1	99.7	94.1**
Histopathological findings:								
Kidneys–								
Medulla mineralization								
% affected	0	0	0	80	10	40	0	30
Average grade	0.00	0.00	0.00	1.13	1.00	1.00	0.00	1.00
Lung –								
Hemorrhage								
% affected	0	10	0	30	10	0	0	0
Average grade	0.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00
Alveolar MΦ accumulation								
% affected	0	0	0	90	0	0	0	60
Average grade	0.00	0.00	0.00	1.11	0.00	0.00	0.00	1.17

= Blood samples collected from 3 rats/sex/group on days 1 and 14 at predose, 0.5, 1, 2, 4, and 8, 12, and 24 hours postdose. Each rat was bled at 3 occasions

* P<0.05 ** P<0.01

For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe. Average grade is calculated as sum of (grade X # of animals with that grade finding)/total # affected.

No-Toxic Effect Level (NOEL): 125 mg/kg/day

There were also a number of mild clinical observations, hematology, clinical chemistry, and histological changes related to drug treatment. Some of them may be attributed to renal toxicities, e.g., stained fur around urogenital area, slight decreases in plasma electrolyte levels and slight increases in the urea nitrogen and creatinine levels, increased kidney weights, and slight mineralization of the renal medulla. The pulmonary histopathological findings were associated with the death of a high dose female that died of moderate pulmonary congestion and/or edema. However, the significance of this toxicity is unclear in an organ that is the site of the influenza viral infection.

The prodrug, Ro 64-0796, was found to be unstable in rodent plasma *ex vivo*. Although it's clear that Ro 64-0796 was present in adult rat plasma (see the urine results from the 6-month study), it is undetectable. Thus, the plasma concentration of the active metabolite, Ro 64-0802,

was really the sum of the prodrug plus the active metabolite. The toxicokinetic data suggested slight accumulation after repeated drug administration. The exposure increased proportionally greater than the dose.

B4. Ro 64-0796/002 (4104): A one month oral (gavage) toxicity and toxicokinetic study in the rat (Report # W-142761; Study # SAR677; Lot # 4104-02-B-4).

Species/Strain: Sprague-Dawley rats	Route: Oral (gavage)	Vehicle: Sterile water	Dose Volume: 10 ml/kg
Weight Range: M = 248-292 g; F = 182-224 g	Duration of Dosing: 4 weeks	Recovery period: 2 weeks	

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Twice daily	Gross pathology	Termination
Body weight	Twice weekly	Organ weights	Termination
Food/water consumption	Twice weekly	Histopathology	Termination on control & high dose at week 4 only
Ophthalmoscopy	Week 4 for control and high dose		
Urinalysis & clinical pathology	Weeks 4 & 6 (recovery animals only)	Toxicokinetics	Days 1 and 29 at 1, 4, 8, and 24 hrs postdose

Important findings

Body weights: There was a dose-related reduction in the body weights in the treated males. Although they only reached statistical significance (8-10% lower than the concurrent controls from days 3-14) in the high dose group (2000 mg/kg/day). Body weights were similar in the females.

Sex	Males				Females			
Daily Dosage (mg/kg/day)	0	50	250	1500	0	50	250	1500
Number of animals:								
Main study	8	8	8	8	8	8	8	8
Toxicokinetic#	2	2	2	2	2	2	2	2
Recovery	2	2	2	2	2	2	2	2
$AUC_{0\rightarrow 24h}$ (µg-h/ml) Day 1	-	11.4	96.3	532	-	9.2	90.7	555
Day 29	-	13.8	98.1	616	-	12.6	112	590
C_{max} (µg/ml) Day 1	-	3.0	17.8	46.2	-	3.32	17.0	51.3
Day 29	-	4.6	13.7	51.3	-	4.82	15.5	56.8
C_{min} (µg/ml) Day 1	-	0.017	0.074	4.59	-	-	0.031	4.89
Day 29	-	0.014	0.159	3.97	-	-	0.201	2.61
T_{max} (h) Day 1	-	1.0	1.0	8.0	-	1.0	2.5	8.0
Day 29	-	1.0	4.0	8.0	-	1.0	2.5	6.0
Clinical observations:								
Salivation (% affected)	0	0	100	100	0	0	100	100
Piloerection (% affected)	0	0	0	16.7	0	0	0	0
Hematology:								
Hemoglobin (g/dl)	151	155	151	150	148	143	147	141**
Packed cell volume	0.386	0.397	0.389	0.384	0.381	0.364**	0.374	0.362**
Red cell distr. width (%)	14.9	14.5	14.3	15.5	14.1	14.7*	14.1	15.2***
Leukocyte (10^9/l)	12.0	11.6	11.7	16.4**	7.1	11.4***	7.8	11.4***
Abs. lymphocytes (10^9/l)	8.87	8.60	8.24	11.09*	5.74	8.50**	6.50	8.39**
Abs. neutrophils (10^9/l)	2.20	1.88	2.62	4.05*	0.96	2.22***	1.16	2.38***
Abs. monocytes (10^9/l)	0.51	0.39	0.42	0.82*	0.16	0.41***	0.25	0.43***
Clinical chemistry:								
Total protein (g/l)	69	69	69	70	75	68***	70***	72*
Sodium (meq/l)	148	148	148	146	148	145**	145**	146*
Phosphate (mmol/l)	1.9	2.2	1.8	2.0	1.3	1.8***	1.9***	1.8***
Urea (mmol/l)	5.1	5.6	5.2	7.2***	7.8	5.9	5.7	6.2

Sex	Males				Females			
Dosage (mg/kg/day)	0	50	250	1500	0	50	250	1500
Number of animals	8	8	8	8	8	8	8	8
Urinalysis:								
pH	7.3	7.1	6.7	6.5**	6.5	6.9	6.3	6.1*
Protein (g/l)	1.08	1.26	1.45	1.50	0.47	0.52	0.42	0.98*
NAG† (IU/ml)	1406	1477	1420	2160***	1376	1306	1407	2182**
Relative organ weights (%):								
Adrenals	0.015	0.018	0.018	0.019*	0.035	0.032	0.032	0.032
Kidneys	0.679	0.711	0.707	0.830***	0.725	0.705	0.708	0.827**
Thymus	0.139	0.125	0.139	0.113	0.189	0.201	0.205	0.158*
Histopathological findings:								
Kidneys–								
Tubular mineralization								
% affected	0	0	0	87.5	62.5	50	87.5	100
Average grade	0.0	0.0	0.0	1.6	1.2	1.0	1.3	3.4
Basophilic tubules								
% affected	12.5	25.0	62.5	100	12.5	12.5	25	100
Average grade	1.0	1.0	1.0	1.6	1.0	1.0	1.0	1.5
Tubular dilation								
% affected	0	0	0	75	0	0	12.5	37.5
Average grade	0.0	0.0	0.0	1.3	0.0	0.0	1.0	1.0
Granular tubular cast								
% affected	0	0	0	87.5	0	0	0	25
Average grade	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0
PAS-positive tubular cast								
% affected	37.5	25.0	25.0	100	12.5	25.0	12.5	25
Average grade	1.0	0.0	1.0	1.6	1.0	1.0	1.0	1.0

= Blood samples collected from 2 rats/sex/group/time point on days 1 and 29 at 1, 4, 8, and 24 hours postdose. Each rat was bled 4 times.

† = N-acetylglucosaminidase

* P<0.05 ** P<0.01 *** P<0.001

For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5=severe. Average grade is calculated as sum of (grade X # of animals with that grade finding)/total # affected.

No-Toxic Effect Level (NOEL): 50 mg/kg/day

The systemic exposure following one month of repeated drug administration increased slightly even as compared to that seen in the 2-week study, indicating slight drug accumulation. The postdose salivation was observed starting during the 2nd week of drug administration. Piloerection was seen in the high dose males only. The major toxicities were those associated with kidneys. There were some changes in the hematological parameters, especially those relating to leukocytic cells. But they were so small that their toxicological significance is unclear. The histological changes were still present after 2-week of drug-free recovery. Different stages of spermatogenesis were also examined in the testes and found to be similar between the control and treated animals.

B5. Ro 64-0796/002 (also known as 4104): A 4-week oral (dietary administration) range-finding and palatability toxicity study in the rat (Report # W-142979; Study # 276/90-D6154; Lot # 80202143). The sponsor originally planned to administer the drug in the 2-year carcinogenicity study via dietary admixture. Thus, this study was performed as a dose-ranging and bridging study.

Species/Strain: Crl:CDBR rats	Route: Oral (dietary administration)	Duration of Dosing: 4 weeks
Weight Range: M = 154-191 g; F = 145-178 g	Age on Day 1: 6 weeks old	

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Daily & detailed weekly exam	Gross pathology	Termination
Body weight	Weekly	Organ weights	Kidneys, spleen, liver, heart, & brain
Food/water consumption	Weekly	Histopathology	Termination on kidneys only
Clinical pathology	Week 4	Toxicokinetics	End of week 4 at 4, 12, 20, 32, 40, and 48
Urinalysis	Week 3		hrs after start of dark phase (6 P.M.)

Important findings

Clinical observations	Increased incidences of fur staining and rough hair coat were apparent in the animals treated with 1500 and 2500 mg/kg/day starting on the second week of treatment.

Sex	Males					Females				
Nominal dosage (mg/kg)	0	250	650	1500	2500	0	250	650	1500	2500
Actual dosage (mg/kg/day)	0	223-251	569-648	1272-1736	1724-2516	0	234-262	618-651	1295-1593	1912-2530
Number of animals:	6	6	6	6	6	6	6	6	6	6
$AUC_{0\rightarrow 24h}$ (µg-h/ml)	-	91.6	282.5	614.9	1095.2	-	90.1	243.1	637.1	1194.8
C_{max} (µg/ml)	-	6.35	17.91	35.31	65.67	-	5.48	15.99	38.43	73.54
C_{min} (µg/ml)	-	2.40	6.33	11.06	25.9	-	1.33	4.37	14.17	21.57
Body wt gain (g): wk 0-4	142.5	125.8	117.1*	108.3***	77.8***	74.1	76.0	71.0	57.9	41.0***
Food consumption (g/wk)	20.9	18.5	18.5	16.4	15.0	13.5	13.9	13.0	11.5	9.0
Clinical chemistry:										
Alk. Phosphatase (U/l)	563	559	627	665	807***	370	408	499	447	459
Sodium (mmol/l)	140	142	144	149***	146**	140	144	142	146*	145
Potassium (mmol/l)	5.7	4.4***	4.2***	4.3***	4.6***	5.1	4.3**	4.3**	4.4*	4.7
Phosphate (mmol/l)	2.8	2.8	2.7	2.4**	2.3***	2.4	2.5	2.4	2.3	2.3
Urinalysis:										
Volume (ml)	4.5	4.5	3.9	2.5*	1.6***	2.8	1.8	2.4	2.1	1.7
Specific gravity (g/ml)	1.031	1.031	1.033	1.037	1.046*	1.044	1.042	1.047	1.042	1.048
Histopath. findings: (Kidneys only)										
Focal nephropathy										
% affected	83.3	83.3	50	83.3	83.3	66.7	83.3	100	100	100
Average grade	1.0	1.0	1.0	1.0	1.0	1.0	1.6	1.5	2.0	2.3
Corticomed. mineral.										
% affected	0	0	0	50	66.7	100	100	100	100	100
Average grade	0.0	0.0	0.0	1.6	2.0	1.8	3.2	3.2	3.8	4.0
Papillary mineralization										
% affected	0	0	0	0	0	0	0	16.7	16.7	16.7
Average grade	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0

= Blood samples collected from 2 rats/sex/group/time point at end of week 4 at 4, 12, 20, 32, 40, and 48 hrs after the start of dark phase (6 P.M.). Each rat was bled 2 times.

† = N-acetylglucosaminidase

* P<0.05 ** P<0.01 *** P<0.001

For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe 5=severe. Average grade is calculated as sum of (grade X # of animals with that grade finding)/total # affected.

No-Toxic Effect Level (NOEL): 50 mg/kg/day

The reduced body weight gain in the treated groups reflected the decrease in food consumption. The drug consumed by the animals generally falls within 10% of the intended dosages except for the first week of drug treatment. The systemic exposure to the active metabolite Ro 64-0802 was similar between gavage and dietary mean of drug delivery, although the decreased food consumption suggested a slight palatability problem.

There was a slight, dose-related increase in the total red blood cell count, mean cell volume, and mean cell hemoglobin. These changes may reflect the compensatory reaction to repeated blood collection for toxicokinetic determination. Kidneys were the only organ examined histologically. Several treatment-related changes were observed, including focal nephropathy and corticomedullary mineralization. The changes in the plasma electrolyte parameters and the rough and stained coat in the 2 highest dose groups may be the manifestation of the observed renal pathology.

B6. Ro 64-0796/002: A 27-week oral (intubation) toxicity study in rats followed by an 8-week recovery period, and a 27-week oral (intubation) plasma concentration study in rats (Report # N-138531 for the doses of 50, 200, & 1000 mg/kg/day & N-138539 for the dose of 100 mg/kg/day; Study #'s 06992 & 06994 for doses of 50, 200, & 1000 mg/kg/day and 07011 & 07012 for the dose of 100 mg/kg/day; Lot #'s 4104-02-F-2, GPM0176, 71121001, 71121002, & 71121003). Studies 06992 and 06994 were done together where doses of 0, 50, 200, and 1000 mg/kg/day were studied. Study 06992 used 20 rats/sex/dose in which 5 animals/sex/dose were allowed to recover for 8 weeks after 27 weeks of drug treatment. Study 06994 was a toxicokinetic study in which 8 animals/sex/dose were used. In addition to toxicokinetic determinations at designated days, serum chemistry and urine drug/prodrug concentrations were also determined on days 189 and 107, respectively and the animals were allowed to recover for 6 months before sacrifice. Only histopathological evaluation of the kidneys was performed for this study. Studies 07011 and 07012 were conducted together where doses of 0 and 100 mg/kg/day were studied. The study designs were identical to those of studies 06992 and 06994.

Species/Strain: Crl:CDBR rats	Route: Oral (gavage)	Vehicle: Sterile water	Dose Volume: 10 ml/kg
Weight Range: M = 173-233g; F = 122-169 g	Duration of Dosing: 27 weeks	Recovery period: 8 & 26 weeks	

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Moribundity/mortality	Daily	Water consump.	Weeks 5, 9, 13, 17, 21, 25, 30, 35
Clinical observation	Weekly	Gross pathology	Termination at weeks 27 & 35
Body weight	Weekly	Organ weights	Termination at weeks 27 & 35
Food consumption	Weekly	Histopathology	Termination at weeks 27 & 35 on control & high dose, kidneys of all rats
Ophthalmic & Neurologic exams	Before, during, & at end of treatment	Toxicokinetics	Days 1, 29, 85, & 190 at 1, 3, 6, 12, and 24 hrs postdose
Urinalysis, Urine chemistry, & Clinical pathology	10 rats/sex/group in weeks 7, 14, 26, 27 & 5 rats/sex/group during weeks 1 & 8 of recovery	Urine drug/pro-drug determin.	Day 107 from 6 rats/sex/group of TK study during an 18-hour period

Important findings										
Sex	Males					Females				
Daily Dosage (mg/kg)	0	50	100	200	1000	0	50	100	200	1000
Number of animals:										
Main study	30	15	15	15	15	30	15	15	15	15
Toxicokinetic#	16	8	8	8	8	16	8	8	8	8
Recovery	10	5	5	5	5	10	5	5	5	5
Number of deaths:	2	1	0	3	3	0	1	0	0	1
Clinical observations: Unkempt anus (% aff.)	0	0	0	5.56	68.4	5.00	0	0	0	50.0
Water cons. (mg/kg/day) Study 06992/Study 07011	36.8/31.7	38.9	33.0	38.5	52.0**	36/27	39	27	35	39

Sex	Males					Females				
Daily Dosage (mg/kg)	0	50	100	200	1000	0	50	100	200	1000
Number of animals:										
Main study	30	15	15	15	15	30	15	15	15	15
Toxicokinetic[a]	16	8	8	8	8	16	8	8	8	8
Recovery	10	5	5	5	5	10	5	5	5	5
$AUC_{0\rightarrow 24h}$ (µg-h/ml)										
Day 1	-	8.83	19.4	49.8	248	-	9.01	20.4	46.9	263
Day 29	-	9.61	28.0	58.9	317	-	8.84	22.6	54.7	278
Day 85	-	14.5	32.5	83.9	451	-	13.2	30.1	77.6	518
Day 190	-	14.7	37.9	83.2	481	-	13.2	35.6	86.8	717
C_{max} (µg/ml)										
Day 1	-	1.92	4.19	10.8	25.6	-	2.89	7.10	9.87	30.1
Day 29	-	2.61	6.26	9.98	32.9	-	2.95	7.46	17.1	29.9
Day 85	-	5.45	8.34	20.4	38.6	-	6.77	9.52	22.8	67.7
Day 190	-	5.16	11.7	15.6	48.2	-	5.80	11.1	22.1	82.2
C_{min} (µg/ml)										
Day 1	-	<0.010	0.0122	0.0188	3.18	-	<0.010	0.0103	0.0211	1.09
Day 29	-	0.0087	0.0041	0.0509	1.99	-	0.0065	0.0054	0.0277	0.840
Day 85	-	0.0162	0.0149	0.142	3.88	-	0.0038	0.0105	0.106	1.65
Day 190	-	0.0107	0.0259	0.0727	1.75	-	0.0067	0.0186	0.0776	1.30
T_{max} (h)										
Day 1	-	1.0	1.0	1.0	6.0	-	1.0	1.0	1.0	6.0
Day 29	-	1.0	1.0	1.0	3.0	-	1.0	1.0	1.0	3.0
Day 85	-	1.0	1.0	1.0	6.0	-	1.0	1.0	1.0	3.0
Day 190	-	1.0	1.0	1.0	6.0	-	1.0	1.0	1.0	3.0
Urinary conc. (mg/ml)										
Ro 64-0796 (ProDrug)	-	0.186	0.268	0.706	1.665	-	0.127	0.235	0.451	1.344
Ro 64-0802	-	0.339	0.708	1.609	3.283	-	0.310	0.597	1.273	4.051
Hematology[†]:										
Platelet (10^9/l)	910/1469	926	1473	939	1039**	930/1452	912	1477	885	918
Red cell distr. width (%)	13.0/18.6	13.4	18.3	13.5	13.9**	12.4/16.3	12.5	16.4	12.7	12.4
Reticulocytes (%)	2.0/2.3	2.1	1.9	2.1	2.2	2.1/2.0	1.8*	1.9	1.9	1.8*
Leukocyte (10^9/l)	7.9/14.5	10.0*	10.6	9.5	10.9*	5.6/7.9	5.4	7.4	5.5	6.6
Abs. seg. neutro. (10^9/l)	1.85/1.13	2.42	1.77*	2.09	3.27*	0.90/1.12	0.86	0.75	1.14	1.54
Abs. lymphocytes (10^9/l)	5.74/8.15	7.07	8.03	6.94	6.83	4.51/6.37	4.37	6.26	4.14	4.76
Abs. monocytes (10^9/l)	0.23/0.47	0.33	0.64	0.34	0.69*	0.07/0.35	0.13	0.33	0.10	0.16
Clinical chemistry[†]:										
Alk. phosphatase (g/l)	73/70	66	71	72	92*	27/29	28	28	51*	49**
Total bilirubin (mg/dl)	0.17/0.17	0.17	0.19	0.16	0.24**	0.21/0.24	0.24	0.23	0.24	0.24
Cholesterol (mg/dl)	71/68	72	67	73	112**	91/101	88	93	93	110*
Total protein (g/dl)	7.0/6.9	7.0	6.8	7.1	7.6**	7.5/7.7	7.8	7.8	8.4**	8.1**
Albumin (g/dl)	4.5/4.5	4.5	4.5	4.6	4.7*	5.6/5.6	5.6	5.8	5.9	5.6
Globulin (g/dl)	2.5/2.3	2.6	2.3	2.5	2.8**	1.9/2.1	2.2*	2.1	2.5**	2.5**
Albumin/globulin ratio	1.81/1.97	1.77	1.96	1.91	1.69	2.99/2.83	2.52**	2.83	2.35**	2.27**
Urea nitrogen (mg/dl)	14.4/12.0	14.5	12.0	14.7	24.5**	11.9/11.8	11.8	12.2	12.4	16.9**
Creatinine (mg/dl)	0.2/0.2	0.2	0.2	0.2	0.3**	0.2/0.3	0.3	0.3	0.3	0.3**
Calcium (mg/dl)	9.8/9.9	9.9	9.9	10.1	10.1	9.9/10.5	10.1	10.6	10.3**	10.4*
Phosphorus (mg/dl)	5.8/6.4	5.6	6.3	5.6	5.7	4.0/5.5	4.3	5.3	4.3	4.8**
Sodium (mmol/l)	151/145	151	145	151	146**	145/144	149*	144	152**	145
Chloride (mmol/l)	108/104	107	104	105*	94**	103/104	106*	104	107**	98
Magnesium (mg/dl)	2.2/2.2	2.2	2.5	2.1	2.6**	2.3/2.5	2.4	2.4	2.5	2.7**

Sex	Males					Females				
Dosage	0	50	100	200	1000	0	50	100	200	1000
Number of animals	30	15	15	15	15	30	15	15	15	15
Urinalysis†:										
pH	7.07/7.50	7.18	7.12**	7.00	6.56**	6.71/6.68	6.74	6.85	6.66	6.95
Calcium (µmol/hr)	0.91/0.92	0.84	0.93	0.94	1.51***	2.89/2.52	1.87*	2.54	2.00	1.54**
Magnesium (µmol /hr)	5.5/6.5	6.1	7.0	6.5	7.7*	6.5/6.2	5.2*	61	4.7*	6.6
Phosphorus (µmol /hr)	36/28	37	34	42	94***	28/26	24	25	30	68***
Sodium (µmol /hr)	24/27	21	16*	15**	43***	20/18	16	13*	19	61***
Chloride (µmol /hr)	20/18	20	11**	18	69***	20/14	19	10	22	73***
Potassium (µmol /hr)	104/82	95	78	85**	114	55/55	48	53	48	81***
NAG‡ (U/l)	749/512	581	553	639	515*	430/559	484	510	435	500
Creatinine (mmol/l)	9.51/7.62	8.42	7.72	7.19	4.30***	3.64/6.24	4.04	5.63	4.12	2.98
NAG/Creatinine ratio	85.7/70.5	82.6	75.6	97.4	122.1**	122/97.7	122.1	96.7	110.3	176.2**
Relative organ weights:										
Main - Liver (%)	2.37/2.37	2.376	2.292	2.562**	2.772**	2.62/2.54	2.642	2.475	2.778	3.005**
Kidneys (%)	0.59/0.57	0.601	0.570	0.627*	0.692**	0.66/0.62	0.680	0.617	0.699*	0.698*
Adrenals (%)	.012/.012	0.012	0.013	0.013	0.016**	.026/.024	.0.025	0.023	0.030	0.030*
Recovery – Kidneys (%)	0.52/0.55	0.575	0.521	0.545	0.652**	0.60/0.59	0.630	0.606	0.641	0.648
Histopath. findings:										
Kidneys–										
Progressive nephropathy										
% affected	27/13	8	27	7	73	13/7	7	13	0	20
Average severity	1.3/1.5	1.0	1.0	2.0	1.6	1.0/2.0	1.0	1.0	0.0	1.0
Tubular vacuolation										
% affected	8/27	31	27	20	27	27/20	33	47	93	100
Average severity	1.0/1.0	1.0	1.0	1.0	1.3	1.0/1.0	1.0	1.0	1.2	1.8
Medullary mineral.										
% affected	0/20	8	13	7	20	0/0	0	13	0	13
Average severity	0/1.0	1.0	1.0	1.0	1.0	0.0/0.0	0.0	1.0	0.0	1.0
Cortical mineralization										
% affected	0/7	0	13	0	47	0/0	0	7	0	0
Average severity	0/1.0	0.0	1.0	0.0	1.0	0.0/0.0	0.0	1.0	0.0	0.0
Cor./medul. Mineral.										
% affected	0/0	8	0	20	67	33/27	20	27	0	33
Average severity	0.0/0.0	1.0	0.0	1.0	1.2	1.0/1.0	1.0	1.0	0.0	1.8
Tubular basophilia										
% affected	40/20	31	20	53	27	0/7	7	7	0	13
Average severity	1.0/1.0	1.0	1.0	1.0	1.3	0.0/1.0	1.0	1.0	0.0	1.5
Lung- Intraalveolar MΦ										
% affected	13/33	0	40	27	33	13/7	7	20	20	27
Average severity	1.0/1.0	0.0	1.0	1.5	1.2	1.5/1.0	1.0	1.0	1.0	1.3

= Blood samples collected from 3 rats/sex/group/time point on days 1, 29, 85, and 190 at 1, 3, 6, 12, and 24 hours postdose. Each rat was bled 3 times.

† = Only the data from Day 182 (week 26) are included for hematology, serum chemistry, and urinalysis parameters. Two control values are shown. The 1st value comes from Study 06992 and should be used to compare those of the dose levels 50, 200, and 1000 mg/kg/day. The 2nd value comes from Study 07011 and should be used to compare those for the dose of 100 mg/kg/day.

‡ = N-acetylglucosaminidase

* P<0.05 ** P<0.01 *** P<0.001

For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked. Average grade is calculated as [sum of (grade * # of animals with that grade finding)]/total # affected.

No-Adverse Effect Level (NOAEL): 100 mg/kg/day

The treatment animals received adequate drug exposures in these studies. The systemic exposure seemed to increase following repeated drug administration since the AUC values for the females were still increasing linearly after more than 190 days of drug treatment.

The main target organ of toxicity associated with chronic administration of Ro 64-0796 was kidney. Most of the toxicological findings were associated with renal toxicity. They included the clinical observation of a yellow- or rust-colored discoloration around the urogenital area that was seen starting after 5 weeks of treatment, ranged from transient to consistent observation among the animals, and did not persist during the recovery period. Other manifestations of renal toxicities were various changes in all of the urinalysis parameters, changes in some serum chemistry parameters that may be affected by renal function, increased kidney weights, and the histopathological changes in the kidneys. Following 8 weeks of drug-free treatment, the toxic changes to the kidneys had shown some improvement.

The accumulation of intraaveolar macrophages was observed also in the 14-day toxicity study. The relative organ weights of liver and adrenal glands were affected significantly without the corresponding histopathological changes. However, none of these changes did persist after recovery. The NOAEL for this study was 100 mg/kg/day.

B7. Ro 64-0796 (also known as 4104): Expert assessment of the kidneys from the rat toxicity studies (Report # W-143032; Study #'s 87900, SAR 677, 06992/07011). The sponsor has obtained a point-by-point review from to provide an expert histopathologic evaluation of the kidneys of rats exposed to Ro 64-0796. He had reviewed kidney slides from all of the rat toxicity studies and concluded that the observed renal histopathological was not due to the direct toxicity of Ro 64-0796 rather associated with the high amount of phosphate in the formulation. He felt that the increased renal tubule mineralization was a high dose effect. Basophilic tubules seen in some lower dose animals were due more to spontaneous chronic progressive nephropathy. In addition, the renal tubule mineralization at the OSOM/ISOM junction caused by Ro 64-0796 was morphologically similar to that encountered with purified diets and high dietary phosphorus, without interstitial inflammatory or fibrous reaction. Thus, he reasoned that at the high dose, the phosphate salt of 64-0796 would negatively influence the dietary calcium/phosphate ratio, leading to the precipitation of calcium phosphate and the subsequent renal tubule mineralization in rats that are very sensitive to such change. The expert pathologist put forth another point in support of his hypothesis: the findings that at the same dose and higher systemic exposure and twice the urine concentrations of active drug in marmosets, no renal changes were observed. The assessment may be reasonable and suggests that the renal changes may be a specie-specific phenomenon. However, if one considers the 2-4 fold higher urinary prodrug concentrations in rats as compared to marmosets and lower solubility of prodrug in free base form (expected to exist in kidneys) as compared to the active metabolite, the tubule mineralization may be due partly to the precipitated prodrug. This possibility poses some clinical significance in patients with low esterase levels (hepatic impaired patients) in a chronic treatment setting (i.e., prophylaxis indication).

B8. Ro 64-0796 (also known as 4104): A 14-day range-finding oral toxicity study in juvenile rats (Report # W-143030; Study 71-53). The animals were from 3 litters/dose. Each litter was reduced to 8 pups (4 males and 4 females) on day 6 after birth.

Species/Strain: Sprague-Dawley rats	Route: Oral (gavage)	Vehicle: Sterile water	Dose Volume: 10 ml/kg
Age at start of dosing: Lactation day 7	Weight range: M=12.2-18.2 g; F=12.5-17.9g		Duration of Dosing: 14 days

Data collected: dams	Frequency/Occasion	Data collected: pups	Frequency/Occasion
Clinical observation	Daily	Clinical observation	Twice daily
Body weight	Gestation days 0, 7, 14, 20; Lactation days 0, 4, 7, 14, 20	Viability	Days 4, 6, 7-21 after birth
		Body weight	Lactation days 0, 4, 7, 10, 14, 18, 21
Food consumption	Gestation days 0-1, 6-7, 13-14, 19-20; Lactation days 0-1, 4-5, 7-8, 14-15, 19-20	Clinical pathology	From 2/sex/dose at termination
		Urinalysis	4 hr samples from 2/sex/litter at term.
		Histopathology	At termination

Important findings – F_1 pups

Sex	Males					Females				
Daily Dosage (mg/kg/day)	0	50	150	500	1000	0	50	150	500	1000
Number of animals:	12	12	12	12	12	12	12	12	12	12
Number of death:	0	0	0	0	8	0	0	0	0	10
Clinical observations: Cyanosis before death	0	0	0	0	3	0	0	0	0	3
Body weight gain: (g)	39.88	39.72	37.09	38.59	34.00	35.82	37.05	35.21	38.16	46.29*

* $P<0.05$

No-Toxic Effect Level (NOEL): 500 mg/kg/day

All but one of the deaths occurred around the administration of the 1st dose. There were some minor differences in the clinical pathology parameters between the control and treated groups. However, since many of the values were from 1 or 2 animals, it's unclear whether these differences have any toxicological significance. Thus, 1000 mg/kg/day exceeded the maximum tolerated dose while the 500 mg/kg/day was the NOAEL.

B9. Ro 64-0796 (also known as [redacted]4104): A two-week oral toxicity study in juvenile rats aged 7-20 days (Report # W-143066; Study [redacted]71-54). The animals for the main study were from 5 litters/dose, for the recovery satellite study were from 1 litter/dose (control and high dose only), and for the toxicokinetic substudy were from 6 litters/dose.

Species/Strain: Unweaned Sprague-Dawley rats	Route: Oral (gavage)	Vehicle: Sterile H_2O	Dose Vol.: 10 ml/kg
Number of dams: 5/dose for main study, 1/dose for recovery, 6/dose for TK study			Duration of Dosing: 14 days
Age at start of dosing: Lactation day 7	Weight range: M=12.2-18.9 g; F=11.5-18.0g		Recovery period: 4 weeks

Data collected: dams	Frequency/Occasion	Data collected: pups	Frequency/Occasion
Clinical observation	Daily	Clinical observation	Twice daily
Body weight	Gestation days 0, 7, 14, 20; Lactation days 0, 4, 7, 14, 20	Viability	Days 4, 6, 7, 21 after birth
		Litter reduction	4/sex on lactation day 6
		Body weight	Lactation days 0, 4, 7, 10, 14, 18, 21
Food consumption	Gestation days 0-1, 6-7, 13-14, 19-20; Lactation days 0-1, 4-5, 7-8, 14-15, 19-20	Hematology	From 6/sex/dose at termination
		Serum biochemistry	From 14/sex/dose at termination
		Urinalysis	4 hr samples from 2/sex/litter at days 19 & 20
		Histopathology	At termination
		Toxicokinetics	Days 1 & 14 at 1, 2, 4, 7, 12, 24 hr postdose

Important findings – F_1 pups									
Sex			Males				Females			
Daily Dosage (mg/kg/day)			0	50	150	500	0	50	150	500
Number of animals:										
Main study			20	20	20	20	20	20	20	20
Recovery			4	0	0	4	4	0	0	4
Toxicokinetic study			0	24	24	24	0	24	24	24
Ro 64-0796-Prodrug	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	8.71	63.99	330.89	-	9.22	73.53	358.16
		Day 14	-	1.21	19.64	60.62	-	1.37	19.74	64.74
	C_{max} (µg/ml)	Day 1	-	5.72	12.30	46.20	-	5.17	15.70	52.10
		Day 14	-	1.47	7.80	16.60	-	1.22	8.79	18.90
	C_{min} (µg/ml)	Day 1	-	0.0542	0.123	0.849	-	0.0991	0.0187	1.010
		Day 14	-	0.0022	0.0187	0.0172	-	0.0022	0.0110	0.0217
	T_{max} (h)	Day 1	-	1.0	2.0	2.0	-	1.0	1.0	2.0
		Day 14	-	1.0	1.0	1.0	-	1.0	1.0	2.0
Ro 64-0802-Metab.	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	8.29	46.88	185.88	-	8.43	64.21	233.49
		Day 14	-	3.55	29.08	78.13	-	3.19	25.39	76.92
	C_{max} (µg/ml)	Day 1	-	1.69	4.63	15.10	-	1.20	6.79	17.90
		Day 14	-	1.36	4.83	9.55	-	0.99	4.47	10.70
	C_{min} (µg/ml)	Day 1	-	0.0729	0.314	1.650	-	0.101	0.298	1.880
		Day 14	-	<0.010	0.0547	0.0373	-	<0.010	0.0300	<0.010
	T_{max} (h)	Day 1	-	2.0	4.0	4.0	-	4.0	4.0	4.0
		Day 14	-	2.0	2.0	4.0	-	2.0	2.0	4.0
Body weight gain: (g) Days 7-20			36.01	35.43	35.91	36.58	33.62	34.10	34.57	36.54*
Serum Biochemistry:										
Total bilirubin (mg/dl)			0.179	0.137*	0.164	0.163	0.194	0.167	0.166	0.139*
Total protein (g/dl)			4.24	4.23	4.23	4.36	4.24	4.33	4.29	4.57**
Albumin (g/dl)			3.41	3.36	3.41	3.49	3.50	3.47	3.44	3.67*
Globulin (g/dl)			0.83	0.87	0.81	0.87	0.74	0.86	0.84	0.90*
Urinalysis:										
Creatinine (mg/dl)			14.22	13.30	10.58	7.46*	13.12	11.97	9.03	7.89
Chloride (meq/l)			30.2	30.7	34.1	32.6	29.0	22.8	24.8	50.0*
Volume (ml) - Day 20			1.11	1.15	1.34	1.78**	1.02	1.04	1.24	1.73**
Potassium (meq)			0.0580	0.0693	0.0524	0.0791	0.0480	0.0433	0.0469	0.0822**
Chloride (meq)			0.0310	0.0362	0.0355	0.0595*	0.0255	0.0218	0.0273	0.0876**
Relative organ weight: (mg/100 g)										
Brain			2741.8	2692.0	2711.3	2550.3	2804.0	2736.6	2696.8	2571.6**
Thymus			480.5	464.0	454.9	425.1**	454.3	472.9	462.2	478.7
Adrenals			32.6	28.4	28.2**	26.85**	33.84	27.8**	27.9**	27.7**
Testes/Uterus			420.6	421.4	382.1*	374.19*	81.1	73.8	68.7*	67.9*

† = Blood samples were collected from 4 rat/sex/dose/time point at 1, 2, 4, 7, 12, & 24 hours postdose. Each animal was bled twice, once on day 1 and once on day 14.

* P<0.05 ** P<0.01

No-Toxic Effect Level (NOEL): 50 mg/kg/day

Initially, the systemic exposure to the prodrug, Ro 64-0796, was higher than that to the active metabolite, Ro 64-0802, suggesting that the neonatal rats did not metabolize the prodrug as efficiently as the adult rats. Following repeated drug administration, the conversion to the metabolite improved while the systemic exposures to both the prodrug and the active metabolite decreased. The systemic drug exposure to the active metabolite in these neonatal rats was lower than that in the adults dosed for the same duration and at the same dosage. The detectable

amount of prodrug in these juvenile rats suggested that their plasma lacks the esterases that cause the *ex vivo* unstability of the prodrug in adult rat plasma.

The high mortality rate at 1000 mg/kg/day dose group observed in the previous range-finding study may be associated with the high amount of unhydrolyzed prodrug. Most of the deaths from that study occurred after the 1st dose where systemic exposure to prodrug exceeded that to the active metabolite. Based on the following study where weaned juvenile rats were used, the ratio of plasma exposures to the prodrug and active metabolite was estimated at 1:3.

There were various changes to serum biochemistry and urinalysis parameters. But since the changes were mostly very slight without the corresponding histopathological findings, the significance of these were unclear.

B10. Ro-0796 (also known as [redacted]4104): A four-week oral toxicity study in juvenile rats aged 21-49 days (Report # W-143067; Study [redacted]1-55).

Species/Strain: Weaned Sprague-Dawley rats	Route: Oral (gavage)	Vehicle: Sterile H_2O	Dose vol.: 10 ml/kg
Age: 21 days old	Weight range: M=43-57 g; F=40-60 g	Duration of dosing: 4 weeks	Recovery period: 4 weeks

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Twice daily	Urinalysis	4 hr samples on day 25 of dosing & day 25 of recovery
Body weight	Twice weekly	Gross autopsy	At termination
Food consumption	Twice weekly	Organ weights	At termination
Hematology	At termination	Histopathology	At termination for control and high dose only
Serum biochemistry	At termination	Toxicokinetics	From 2 rats/sex/dose/time point at 1, 2, 4, 7, 12, and 24 hours postdose on days 4 & 27

Important findings

Sex			Males				Females			
Daily Dosage (mg/kg/day)			0	50	150	500	0	50	150	500
Number of animals:										
Main study			20	20	20	20	20	20	20	20
Recovery			4	0	0	4	4	0	0	4
Toxicokinetic study†			0	18	18	18	0	18	18	18
Ro 64-0796-Prodrug	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 4	-	0.563	4.65	23.22	-	0.60	5.28	19.84
		Day 28	-	1.12	8.05	36.41	-	1.16	8.52	43.27
	C_{max} (µg/ml)	Day 4	-	0.45	2.32	5.76	-	0.42	2.15	6.21
		Day 28	-	1.10	3.47	7.16	-	0.67	3.20	9.73
	C_{min} (µg/ml)	Day 4	-	0.00051	0.00151	0.00715	-	<0.001	0.00122	0.00586
		Day 28	-	0.00923	0.0160	0.0126	-	0.0150	0.00647	0.0616
	T_{max} (h)	Day 4	-	1.0	1.0	1.0	-	1.0	1.0	1.0
		Day 28	-	1.0	1.0	2.0	-	1.0	1.0	1.0
Ro 64-0802-Metab.	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 4	-	2.17	18.93	72.84	-	2.08	16.03	75.64
		Day 28	-	4.02	26.51	116.47	-	4.06	29.08	99.13
	C_{max} (µg/ml)	Day 4	-	1.02	4.56	11.50	-	0.97	3.57	14.10
		Day 28	-	1.19	7.28	18.30	-	1.79	6.61	12.50
	C_{min} (µg/ml)	Day 4	-	<0.010	<0.010	0.0323	-	<0.010	<0.010	0.0211
		Day 28	-	0.0275	0.0566	0.0747	-	0.0275	0.00636	0.309
	T_{max} (h)	Day 4	-	1.0	1.0	1.0	-	2.0	1.0	4.0
		Day 28	-	2.0	2.0	2.0	-	1.0	2.0	2.0
Hematology:										
Erythrocytes (10^4/mm^3)			675.6	738.7**	718.9**	708.3*	714.4	734.4	773.7**	751.7*

Sex	Males				Females			
Daily Dosage (mg/kg/day)	0	50	150	500	0	50	150	500
Number of animals:	20	20	20	20	20	20	20	20
Serum Biochemistry:								
Inorganic phosphate (mg/dl)	9.96	10.61	10.55*	10.61*	8.90	8.75	9.52*	9.65**
Calcium (mg/dl)	9.48	9.76	9.88*	9.99**	9.61	9.59	9.99**	9.90*
Magnesium (mg/dl)	2.89	2.96	3.04	3.08	3.09	2.95	3.11*	3.49**
Sodium (meq/l)	145.4	145.4	147.3	150.0*	144.7	145.2	144.8	149.5**
Potassium (meq/l)	4.24	4.04	4.43	4.33	3.86	4.09	4.35**	4.21**
Globulin (g/dl)	1.41	1.37	1.40	1.41	1.30	1.32	1.46**	1.44*
A/G ratio	2.77	2.89	2.81	2.81	3.17	3.06	2.88*	2.81**
Urinalysis:								
Volume (ml)	2.93	3.21	3.71	5.89**	2.54	3.11	3.68*	5.62**
Creatinine (mg/dl)	42.65	33.22	32.30	26.54**	42.81	32.87	32.12	20.83**
Inorganic phosphate (mg/dl)	102.88	97.90	103.91	155.44**	128.15	91.99	114.92	131.70
Sodium (meq/l)	41.8	44.4	69.1*	134.8*	29.8	31.6	47.7**	97.8**
Potassium (meq/l)	190.0	174.5	190.4	142.3*	141.2	114.2	123.3	95.9**
Chloride (meq/l)	72.1	72.3	111.5**	162.9**	47.8	50.7	78.7**	127.2**
NAG# (u/l)	18.53	17.38	17.44	12.00**	14.55	9.57	9.34	6.90**
Calcium (mg)	0.15	0.13	0.12	0.28**	0.15	0.13	0.18	0.25**
Magnesium (mg)	1.03	0.76	0.86	1.91**	0.93	0.84	0.99	2.48**
Sodium (meq)	0.120	0.141	0.231**	0.755**	0.068	0.094	0.177**	0.543**
Potassium (meq)	0.517	0.511	0.637*	0.758**	0.312	0.333	0.421**	0.494**
Chloride (meq)	0.199	0.222	0.378**	0.896**	0.112	0.152	0.279**	0.700**

= N-acetylglucosaminidase

† = Blood samples were collected from 3 rats/sex/dose/time point at 1, 2, 4, 7, 12, & 24 hours postdose. Each animal was bled twice: once on day 4 and once on day 28.

* P<0.05 ** P<0.01

No-Toxic Effect Level (NOEL): None

The pharmacokinetics in these slightly more mature juvenile rats was more similar to that in the adult rats. Since the *ex vivo* Ro 64-0796 concentrations in adult rat plasma cannot be measured, it is assumed that the pharmacokinetic values for prodrug in adults will be similar to those in these juvenile rats.

There were various small but statistically significant serum chemistry and urinalysis changes without the corresponding histopathological findings in liver and kidneys. Red blood cells were also slightly but significantly increased at mid and the high dose groups. These changes were so slight, their toxicological significance was unclear.

B11. Ro 64-0802/002: A 2 week intravenous toxicity and toxicokinetic study in the rat (Report # W-142938; Study # SAR691; Lot # 4071-01-A-1).

Species/Strain: Male Sprague-Dawley rats		Route: Intravenous		Vehicle: Isotonic saline
Weight range: 202-241g		Duration of dosing: 2 weeks		Dose vol.: 10 ml/kg
Data collected	**Frequency/Occasion**	**Data collected**	**Frequency/Occasion**	
Clinical observation	Twice daily	Gross autopsy	At termination	
Body weight	Twice weekly	Organ weights	At termination including brain, etc.	
Water consumption	Predose, once during dosing	Histopathology	At termination for control and high dose only	
Hematology	Week 2	Toxicokinetics	From 2 rats/sex/dose/time point at 0.25, 0.5, 1,	
Serum biochemistry	Week 2		3, 5, and 24 hours postdose on day 1 so that	
Urinalysis	Overnight samples at week 2		each animal was bled 3 times	

Important findings				
Daily Dosage (mg/kg/day)	0	5	20	100
Number of animals:				
Main study	6	6	6	6
Toxicokinetic study	4	4	4	4
$AUC_{0-\infty}$ (µg-h/ml)	-	1.84	8.55	53.9
Clearance (l/hr/kg)	-	2.7	2.3	1.9
V_{ss} (l/kg)	-	3.2	5.2	2.6
$T_{½}$ (1) (hr)	-	0.077	0.20	0.19
$T_{½}$ (2) (hr)	-	1.4	4.4	3.4
C_0 (µg/ml)	-	5.62	24.8	157
Histopathological findings:				
Injection site – Inflammatory cell infiltration				
% affected	100	-	-	83.3
Average severity	1.5	-	-	2.0
Exudate/necrosis				
% affected	50.0	-	-	83.3
Average severity	1.3	-	-	1.8
Lung – Alveolitis				
% affected	0	-	-	50.0
Average severity	0.0	-	-	1.7

* P<0.05 ** P<0.01

For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked. Average grade is calculated as [sum of (grade * # of animals with that grade finding)]/total # affected.

No-Toxic Effect Level (NOEL): None

The exposure at 100 mg/kg by intravenous injection was equivalent to that at 200 mg/kg/day by oral administration. Consistent with results in previous studies, very mild toxicity was observed at this systemic exposure. Although inflammatory reactions at injection site were seen in both control and the high dose animals, the presence of the drug seem to exacerbate the severity of the reaction. Alveolitis has been associated with the drug treatment in previous studies by oral administration.

B12. Ro 64-0796/001: A preliminary 7 day oral toxicity and toxicokinetic study in the marmoset (Report # W-142646; Study # SAP668E; Lot # 1029-92).

Species/Strain: Marmosets	Route: Oral (intragastric)	Vehicle: Carbomethyl cellulose/Tween 80	
Age: 16-23 months old	Weight range: 318-469g	Duration of dosing: 7 days	Dose volume: 10 ml/kg

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Frequently	Gross autopsy	At termination
Body weight	Weekly	Organ weights	At termination
Hematology	At termination	Histopathology	At termination
Serum biochemistry	At termination	Toxicokinetics	At 1, 3, 7 and 24 hours postdose on days 1 &
Urinalysis	Overnight samples before term.		7 for all animals including controls

Important findings										
Sex			Males					Females			
Daily Dosage# (mg/kg/day)			0	100	500	1000	2000†	0	100	500	2000
Number of animals:			2	2	2	4	2	2	2	2	2
Ro 64-0796-Prodrug	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	8.55	31.25	25.40	18.30	-	7.95	51.75	34.70
		Day 7	-	11.15	71.75	110.98	-	-	11.00	66.60	-
	C_{max} (µg/ml)	Day 1	-	4.1	6.55	4.94	6.56	-	3.66	11.89	16.1
		Day 7	-	2.91	13.54	14.92	-	-	2.60	16.38	-
	T_{max} (h)	Day 1	-	1.0	2.0	1.0	2.0	-	1.0	1.0	1.0
		Day 7	-	1.0	2.0	2.5	-	-	3.0	3.0	-
Ro 64-0802-Metab.	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	67.15	226.95	317.48	357.5	-	63.95	238.70	492.2
		Day 7	-	56.9	312.70	719.45	-	-	32.9	277.05	-
	C_{max} (µg/ml)	Day 1	-	17.53	44.09	52.02	59.64	-	14.84	38.90	92.06
		Day 7	-	14.17	42.13	65.49	-	-	7.69	43.66	-
	T_{max} (h)	Day 1	-	1.0	2.0	1.5	2.0	-	2.0	2.0	1.0
		Day 7	-	3.0	3.0	4.0	-	-	3.0	3.0	-
Intercurrent mortality			0	0	0	0	0	0	0	0	1
Histopathological findings:											
Kidneys–Tubular dilation											
% affected			50	-	-	100	100	50	-	-	100
Average severity			1.0	-	-	1.5	1.0	2.0	-	-	2.0
Stomach–Subchronic inflammation											
% affected			0	0	0	0	100	0	-	0	100
Average severity			0.0	0.0	0.0	0.0	2.0	0.0	-	0.0	1.0
Mucosal atrophy											
% affected			0	0	0	25	100	0	-	0	50
Average severity			0.0	0.0	0.0	3.0	2.5	0.0	-	0.0	2.0
Submucosal edema											
% affected			0	0	0	0	50	0	-	0	0
Average severity			0.0	0.0	0.0	0.0	3.0	0.0	-	0.0	0.0
Mucosal hemorrhage											
% affected			0	0	0	0	100	0	-	0	100
Average severity			0.0	0.0	0.0	0.0	1.5	0.0	-	0.0	2.0
Submucosal hemorrhage											
% affected			0	0	0	0	50	0	-	0	0
Average severity			0.0	0.0	0.0	0.0	2.0	0.0	-	0.0	0.0
Mucosal erosion											
% affected			0	0	0	0	100	0	-	0	100
Average severity			0.0	0.0	0.0	0.0	2.5	0.0	-	0.0	2.5
Mucosal ulceration											
% affected			0	0	0	0	50	0	-	0	0
Average severity			0.0	0.0	0.0	0.0	3.0	0.0	-	0.0	0.0

= The daily dose was split into 2 subdoses given 2 hours apart. Single daily doses were given on the 1st 4 days of dosing at 0, 100, and 500 mg/kg/day, the 1st day at 1000 mg/kg/day, and both days at 2000 mg/kg/day.
† = Dosing at this level was stopped after 2 days and the rest of the animals sacrificed on day 4.

No-Toxic Effect Level (NOEL): 100 mg/kg/day

The frequency and severity of vomiting and/or excessive salivation was observed in a dose-dependent manner, starting at a dose level of 500 mg/kg/day. One female dosed at 2000 mg/kg/day had to be sacrificed *in extremis* because of excessive irritation (marked mucosal hemorrhage, erosion, ulceration, and atrophy) to the gastrointestinal tract. Once the single daily

dose was split into 2 subdoses given 2 hours apart, the frequency and severity of vomiting was reduced.

There was some sex-related difference in the toxicokinetic parameters and females had higher systemic drug exposure. However, the difference was less than one-fold and may not be statistically significant.

B13. Ro 64-0796 (also known as /4104): 28-day oral (gavage) study in the marmoset monkey with a 14 day treatment-free period (Report # RR W-142697; Study # 96-TOX-4104-003; Lot # 1132-5-14).

Species/Strain: Marmosets	Route: Oral (gavage)	Vehicle: Distill H_2O	Weight range: M=310-470g; F=230-440g
Age: 1-5 years old	Dose volume: 10 ml/kg/dose	Duration of dosing: 28 days	Recovery period: 14 days

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Twice daily	Gross autopsy	At termination
Body weight	Daily	Organ weights	At termination
Food consumption	Daily	Histopathology	At termination on control and high dose only
Electrocardiography	At termination	Toxicokinetics	At predose, 3, 5, 8 and 14 hrs after 1st split dose on days 1, 28, & 30
Clinical pathology	At termination		

Important findings

Sex			Males				Females			
Daily Dosage* (mg/kg/day)			2 X 0	2 X 50	2 X 150	2 X 500	2 X 0	2 X 50	2 X 150	2 X 500
Number of animals:										
Main study			4	4	4	4	4	4	4	4
Recovery			2	0	0	2	2	0	0	2
Toxicokinetic			0	2	2	2	0	0	0	2
Ro 64-0796-Prodrug	$AUC_{0\rightarrow\infty}$ (µg-h/ml)	Day 1	-	11.4	55.8	83.8	-	-	-	97.3
	$AUC_{0\rightarrow24h}$ (µg-h/ml)	Day 28	-	8.33	26.6	69.7	-	-	-	89.3
	C_{max} (µg/ml)	Day 1	-	2.87	13.20	10.10	-	-	-	13.10
		Day 28	-	2.62	4.02	14.10	-	-	-	10.50
	T_{max} (h)	Day 1	-	3	3	5	-	-	-	3
		Day 28	-	3	3	3	-	-	-	3
Ro 64-0802-Metab.	$AUC_{0\rightarrow\infty}$ (µg-h/ml)	Day 1	-	40.3	176	614	-	-	-	456
	$AUC_{0\rightarrow24h}$ (µg-h/ml)	Day 28	-	47.0	212	765	-	-	-	920
	C_{max} (µg/ml)	Day 1	-	9.73	26.40	86.80	-	-	-	60.20
		Day 28	-	8.28	27.00	125.00	-	-	-	75.40
	T_{max} (h)	Day 1	-	3	3	5	-	-	-	3
		Day 28	-	3	3	5	-	-	-	5
Clinical Observations: (% affected)										
Massive salivation before 1st dose			0	0	0	62.5	0	0	0	0
Massive salivation after 1st dose			0	0	100	100	0	0	100	100
Massive salivation before 2nd dose			0	0	33.3	75	0	0	0	50
Massive salivation after 2nd dose			0	0	83.3	87.5	0	0	75	87.5
Emesis after 2nd dose			0	0	0	25	0	0	0	25
Wound at angle of the mouth			0	0	0	37.5	0	0	0	62.5

No-Toxic Effect Level (NOEL): 100 mg/kg/day

Salivation and emesis were associated with the administration of Ro 64-0796. No other effects were associated with the treatment. Repeated exposure to the drug seems to increase the systemic drug exposure, suggesting drug accumulation.

B14. Ro 64-0796/002: A 9 month oral toxicity and toxicokinetic study in the marmoset (Report # W-143011; Study # SCP681; Lot # 4104-02-F2).

Species/Strain: Marmosets	Route: Oral (gavage)	Vehicle: Acetate buffer, pH 4.0	Weight range: 286-479 g
Age: 19-24 months old	Dose volume: 10 ml/kg	Duration of dosing: 9 months	Recovery period: 9 weeks

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Twice daily	Urinalysis	Overnight samples at pre-study, weeks 8, 13, 19, 25, 38, & end of recovery
Body weight	Weekly		
H_2O consumption	5day periods in weeks 2, 14, 18, 24, 32, 37, & 45	Gross autopsy	At termination
		Organ weights	At termination
Ophthalmoscopy	Pre-study, weeks 8, 13, 26, 38	Histopathology	At termination on control and high dose only
Electrocardiography	Pre-study, weeks 24, 38, & 48	Toxicokinetics	At 0.5, 1, 2, 3, 7, 12 and 24 hrs after 1st split dose on days 1, 56, 91, 176, & 267
Clinical pathology	Weeks 8, 13, 19, 25, 38, & 48		

Important findings

Sex		Males				Females			
Daily Dosage[a] (mg/kg/day)		2 X 0	2 X 25	2 X 100	2 X 500	2 X 0	2 X 25	2 X 100	2 X 500
Number of animals:									
Main study		3	3	3	3	3	3	3	3
Recovery		2	2	2	2	2	2	2	2
Intercurrent mortality		0	0	2	0	0	1	0	0
Clinical Observations: (% affected)									
Increased salivation		0	0	0	100	0	0	0	100
Emesis due to dosing		0	0	0	100	0	0	0	100
Urinalysis:									
Chloride (mmol/l/hr)	Week 13	11.6	10.8	2.6**	2.8**	11.2	6.2	7.9	3.4*
	Week 19	5.9	7.9	2.2**	2.2**	5.4	5.9	5.7	2.6
	Week 25	9.1	7.5	2.9*	2.6*	9.9	7.7	7.9	4.0
	Week 38	5.7	4.8	4.0	2.1**	5.7	5.3	6.9	2.5*
Calcium (mmol/l/hr)	Week 25	0.12	0.19	0.08	0.07**	0.82	0.11	0.13	0.10
	Week 38	0.17	0.18	1.83	2.55*	0.07	0.08	1.47	1.04
Magnesium (mmol/l/hr)	Week 38	0.73	0.77	0.78	0.43**	0.46	0.66	0.78	0.27
Ro 64-0796-Prodrug T_{max} (h)	Day 1	-	1.0	0.5	0.5	-	3.0	3.0	0.5
	Day 56	-	3.0	3.0	3.0	-	3.0	0.5	3.0
	Day 91	-	0.5	0.5	3.0	-	0.5	0.5	1.0
	Day 176	-	1.0	3.0	0.5	-	3.0	1.0	3.0
	Day 267	-	0.5	0.5	0.5	-	3.0	0.5	1.0
C_{max} (µg/ml)	Day 1	-	1.04	2.81	9.00	-	1.91	3.31	9.58
	Day 56	-	1.65	5.05	10.60	-	2.70	3.53	10.50
	Day 91	-	1.06	4.84	9.66	-	1.96	3.10	10.90
	Day 176	-	1.13	4.33	10.60	-	1.31	5.08	12.10
	Day 267	-	1.70	3.52	12.60	-	1.74	2.81	11.70
C_{min} (µg/ml)	Day 1	-	0.0032	0.0025	0.263	-	0.0037	0.0244	0.216
	Day 56	-	0.0058	0.0025	0.0664	-	0.0138	0.0024	0.0774
	Day 91	-	0.0028	0.0021	0.0712	-	0.0079	0.0078	0.143
	Day 176	-	0.0026	0.0049	0.0238	-	0.0128	0.0061	0.177
	Day 267	-	0.0037	0.0025	0.106	-	0	0.0030	0.144
$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	2.88	11.25	45.33	-	7.30	14.55	50.08
	Day 56	-	5.78	19.95	51.18	-	9.41	14.88	50.43
	Day 91	-	2.78	23.08	58.79	-	7.06	13.15	54.30
	Day 176	-	2.64	17.64	52.40	-	6.43	15.02	65.74
	Day 267	-	3.68	12.87	62.07	-	5.80	7.87	97.51
Urinary concentration[a] (µg/ml)		-	22.7	74.4	470	-	43.8	80.0	727

Sex			Males				Females			
Daily Dosage# (mg/kg/day)			2 X 0	2 X 25	2 X 100	2 X 500	2 X 0	2 X 25	2 X 100	2 X 500
Number of animals:										
	Main study		3	3	3	3	3	3	3	3
	Recovery		2	2	2	2	2	2	2	2
Ro 64-0802-Metab.	T_{max} (h)	Day 1	-	3.0	3.0	3.0	-	3.0	3.0	3.0
		Day 56	-	1.0	1.0	3.0	-	1.0	3.0	2.0
		Day 91	-	3.0	3.0	3.0	-	3.0	3.0	3.0
		Day 176	-	3.0	3.0	3.0	-	1.0	3.0	3.0
		Day 267	-	3.0	3.0	3.0	-	3.0	3.0	7.0
	C_{max} (µg/ml)	Day 1	-	9.65	29.20	101.00	-	6.30	22.00	57.60
		Day 56	-	6.00	21.60	98.10	-	4.29	25.10	53.10
		Day 91	-	9.14	39.20	106.00	-	5.52	24.30	76.90
		Day 176	-	7.02	36.30	127.00	-	4.20	21.00	109.00
		Day 267	-	8.49	32.30	84.10	-	4.16	18.60	170.00
	C_{min} (µg/ml)	Day 1	-	0.0336	0.0821	1.160	-	0.0591	0.123	1.460
		Day 56	-	0.0256	0.196	1.240	-	0.123	0.0901	1.100
		Day 91	-	0.0352	0.0791	1.890	-	0.0772	0.161	1.310
		Day 176	-	0.0589	0.214	1.100	-	0.172	0.151	1.470
		Day 267	-	0.0419	0.129	2.230	-	0.0243	0.114	1.590
	$AUC_{0\rightarrow 24h}$ (µg-h/ml)	Day 1	-	41.58	144.2	469.1	-	26.56	111.1	509.6
		Day 56	-	26.26	116.6	527.8	-	20.81	119.3	372.4
		Day 91	-	17.18	181.0	678.8	-	11.25	123.8	511.4
		Day 176	-	32.68	183.3	675.2	-	24.97	119.2	738.8
		Day 267	-	38.28	123.6	613.4	-	20.78	102.1	1182.2
	Urinary concentration# (µg/ml)		-	287	1250	8730	-	490	2410	10500

= Urinary concentrations of Ro 64-0796 and Ro 64-0802 were measured from 2 animals after ~ 3 months of dosing with calculated recoveries over 18 hours.

* P<0.05 ** P≤0.01

No-Toxic Effect Level (NOEL): 200 mg/kg/day

Except for drug induced salivation and emesis at the high dose group, very few effects were related to the drug treatment. There were statistically significant decrease in the secretion rate of chloride, calcium, and magnesium, most notably in males. However, these changes were small and not accompanied by alterations in serum chemistry parameters or renal and/or hepatic histopathology.

As stated previously, the urinary concentration of the active metabolite, Ro 64-0802, was twice that in rats without the associated renal histopathological changes. However, the urinary concentration of the prodrug, Ro 64-0796, in the marmoset was 2-4 fold less than that in rats while the systemic drug exposure in rats and marmoset at the same dose was comparable, albeit slightly higher in marmosets. This result raised the possibility that a lack of renal effect in marmoset may be partly due to lower renal exposure to the prodrug. The solubility of prodrug in free base form is expected to be 100-1000 fold lower than the phosphate salt form. This possibility raised a concern for humans with lower amount of esterases, thus, higher concentration of prodrug. Since both prodrug and the active metabolite are cleared renally, high plasma prodrug concentration in patients may induce tubule mineralization observed in rats.

The AUC value for the active metabolite increased one-fold following 9 months of repeated drug administration, suggesting some drug accumulation. The systemic exposure level for the prodrug also increased but to a lesser extent.

There were 3 intercurrent mortalities, all of them related to osteomalacia. They were deemed not related to the drug treatment since no high dose or any other animal was affected and alkaline phosphatase levels between control and all other dosing groups were comparable. This may be a reasonable assessment for this particular study, however, taken together with various bone abnormalities in the fetuses exposed to Ro 64-0796 *in utero* (see the studies under "Reproductive Toxicology" section), it is suggested that Ro 64-0796 may have some effects on the ossification process.

B15. Ro 64-0802/002: A 14 day intravenous repeated dose toxicity study of Ro 64-0802/002 in male marmoset (Report # W-142940; Study ____ 71-51; Lot # 4071-01-A-1).

Species/Strain: Male marmosets	**Route:** Intravenous	**Vehicle:** Isotonic saline	**Duration of dosing:** 14 days
Weight range: 276-372g	**Age:** 16-26 months old	**Dose vol.:** 10 ml/kg/day	**Infusion rate:** 2 ml/min.

Data collected	**Frequency/Occasion**	**Data collected**	**Frequency/Occasion**
Clinical observation	Twice daily	Gross autopsy	At termination
Body weight	Predose, days 7 and 14	Organ weights	At termination including brain, etc.
Water consumption	Daily	Histopathology	At termination
Food consumption	Daily	Toxicokinetics	From 2 monkeys/sex/dose at 0.25, 0.5, 1, 3, 5, and 24 hours postdose following the 1st dose
Clinical Pathology	Predose, days 2 & 14		
Urinalysis	16-hr samples at days 2 & 9		

Important findings				
Daily Dosage (mg/kg/day)	0	5	15	50
Number of animals:				
Main study	5	3	3	3
Toxicokinetic study#	0	2	2	2
$AUC_{0-\infty}$ (µg-h/ml)	-	5.39	16.5	84.0
Clearance (l/hr/kg)	-	0.93	0.91	0.60
V_{ss} (l/kg)	-	0.36	0.73	0.49
$T_{½}$ (1) (hr)	-	0.33	0.30	0.34
$T_{½}$ (2) (hr)	-	-	6.5	4.5
C_0 (µg/ml)	-	15.2	41.8	140
#: Animals assigned to toxicokinetic groups received only a single dose of the drug.				
No-Toxic Effect Level (NOEL): 50 mg/kg/day				

The exposure at 50 mg/kg by intravenous injection was comparable to that at ~ 100 mg/kg/day by the oral route of administration. There were no drug-related effects. No local irritation was reported for this study, contrary to the injection site inflammation seen in rats receiving intravenous doses of Ro 64-0802.

Overall Toxicology Summary:

Acute, subacute, and subchronic toxicology studies have been carried out in adult and juvenile rats, mice, and marmosets via both oral and intravenous routes of administration. Ro 64-0796 and its major active metabolite, Ro 64-0802, have been found to cause mild toxicities at fairly high dosages. The toxicities to the target organs are described below.

Kidneys: The most prominent toxicities in all species studies were those associated with kidneys. Slight imbalance (less than 1-fold as compared to the controls) of both plasma and urine electrolytes in addition to alterations in other clinical chemistry parameters (e.g., plasma urea nitrogen and creatinine levels) indicative of renal dysfunction were associated with doses greater or equal to 1000 mg/kg/day in mice, rats, and monkeys. Many of these clinical chemistry and urinalysis effects were seen in one sex only with no clear preference in either sex. In rodents, histopathological findings included chronic progressive nephropathy, corticomedullary mineralization, tubular mineralization (seen only in the one-month rat study), tubular vacuolation, basophilic tubules, and focal nephropathy. Incidence and severity of some of these changes increased at a dose-dependent manner. However, interestingly, these renal changes were graded between minimal to slight in severity after 6 months of repeated drug administration at a dose of 1000 mg/kg/day in rats (~300X and 40X human exposure to Ro 64-0796 and Ro 64-0802, respectively). No histopathological changes were associated with Ro 64-0796 administration in marmosets. Increased incidence and severity of mineralization in kidneys may account for the increased relative kidney weight in rats. It is clear that the prolonged and repeated exposure to Ro 64-0796 and its active metabolite causes slight renal dysfunction at fairly high dosages (> 300X and 40X of expected clinical exposure) in all nonclinical species studied. Since most of the histopathological changes were seen in rats only, the sponsor had postulated that mineralization seen in rats was a species-specific finding. It was argued that at the high dose, the high content of phosphate in the Ro 64-0796/002 (a phosphate salt) would negatively influence the dietary calcium/phosphate ratio in a species known to be sensitive to this kind of change. This led to the precipitation of calcium phosphate and the subsequent mineralization in several of renal structures. However, since the sponsor did not offer concrete evidence that the mineral casts/crystals were unequivaocally precipitated calcium phosphate, another possibility also exists. The pharmacokinetic data have indicated that rodents do not hydrolyze Ro 64-0796 to its active metabolite, Ro 64-0802, as efficiently as primates. The urine prodrug/active metabolite ratios for rats and marmosets were 1:3 and 1:15, respectively. Since the free base form of prodrug (the form expected to exist in kidneys) is expected to be 100-1000 times less soluble than the active metabolite, the mineralization in the high dose

rats may be partly due to the precipitation of the prodrug. This scenario may have some clinical implications in severely hepatic impaired patients. All of the renal changes were reversible after a period of drug-free recovery period.

GI system: Ro 64-0796/002 was extremely irritating to the GI tract of marmosets. Emesis and salivation were associated with doses greater than 150 mg/kg. Slightly to moderately severe gastric mucosal inflammation, atrophy, hemorrhage, erosion, and ulceration were associated with doses of 1000 mg/kg. One animal dosed with 2000 mg/kg had to be sacrificed *in extremis* because of severe gastric irritation. The drug had to be administered as 2 separate daily doses to reduce the drug-induced emesis and gastric irritation. In clinical trials, vomiting, nausea, and abdominal pain occurred at 2-3 time higher number of patients receiving 75 mg b.i.d. Ro 64-0796.

Liver: The toxicities to this organ were mild and consisted of slightly increased liver weight and plasma levels of glucose, cholesterol, total protein, globulin, and albumin. All of the increases were less than 1-fold greater than the corresponding controls. There were no hepatic histopathological changes associated with drug treatment. All of the observed changes were reversible upon cessation of drug administration.

Hematology: The toxicities involved, in general, were small (<50%) but statistically significant changes in various red and white cell parameters. Since most of the changes occurred in one or the other sex per study without any apparent pattern, it is unclear of their clinical significance.

Bone: There were 3 intercurrent mortalities in the 9 month toxicity study in marmosets related to osteomalacia. These deaths were not dose-related and occurred in the low and mid dose groups only. The sponsor thus attributed the effect to the pre-existing osteomalacia and believed that supplementing vitamin D in the diet may have prevented these deaths. Slightly but statistically significant elevation of alkaline phosphatase levels were detected in high dose (≥ 1000 mg/kg/day) in a few rat toxicity studies. In the Segment II reproductive studies, incomplete or no ossification of various bones was observed in both rats and rabbit fetuses exposed to Ro 64-0796 *in utero*. Thus, the data suggest that Ro 64-0796 and/or its active metabolite may have some effect on the ossification process.

Lung: Pulmonary alveolitis was seen in several toxicity studies in rats, including the 6-month study. The incidence and severity at the high dose (≥ 1000 mg/kg/day) were slightly higher than the control. Since lung is one of the main site of influenza viral infection, this finding may

affected. In addition, endpoints of parturition can only be measured at one time point. Thus, the overall score for "Signal Strength, Part A" was -1. The prolonged parturition time was first observed at 250 mg/kg/day dose which is probably a good estimate of TD_{10} (the toxic dose where 10% of animals are affected). The clinical dose of 75 mg b.i.d was assumed to be ED_{90} (the dose where 90% efficacy is observed). The systemic exposure was equivalent to that at 50 mg/kg/day in rats. Dividing 250 by 50, the TI (therapeutic index) is estimated to be 5. Ro 64-0796 and its active metabolite are designed specifically as the influenza viral neuraminidase inhibitor and are not expected to affect any of the parturition parameters. Hence, the overall score for effects on "Pharmacodynamics" is 0. It has been stated in the beginning that the metabolic, drug disposition, and general toxicity profiles between human and test species (rats and rabbits) are fairly similar. Thus, the overall score for "Concordance Between the Test Species and Humans" should be 1. The score for "Relative Exposures" is 0 since this value (13; i.e., fold over human exposure) is between 10 and 25. And one other approved influenza neuraminidase inhibitor did not affect any of parturition parameters and would give a score of 0 for "Class Alert." The sum of all of the scores was 0 indicating that this effect should be of low concern to humans, and was not placed into the Label.

Lactation: The affected lactation endpoints included increased incidence of pup without milk in the stomach, increased number of dams that failed to nurse the offspring, and increased pup mortality during the first 4 days postpartum in rats. These effects were associated with doses ≥ 1500 mg/kg/day. At 500 mg/kg/day, no effect on lactation was observed. The score for "Signal Strength Part A" was 1 since multiple lactation endpoints were affected. However, the score for "Signal Strength, Part B" should be -1 since all of the effects occurred at a maternally toxic dose without any dose-response relationship. The score for the "Pharmacodynamics" should be -1 since the TI value is estimated to be 30 (1500 mg/kg/day ÷ 50 mg/kg/day) and the pharmacological and toxicological mechanisms for the effects on lactation were not expected to be similar. The scores for the categories of "Class Alert" and "Concordance Between the Test Species and Humans" were 0 and 1, respectively, as stated previously. Finally, the score for "Relative Exposure" is -1 since at 1500 mg/kg/day, the systemic exposure for the active metabolite was ~ 100X that at recommended clinical dosage. Hence, the total score for changes to the lactation process is -1, indicating that it will pose a low concern as a human reproductive risk.

Developmental Mortality: Increased incidence of postimplantation loss and abortion in rabbits (at a dose of 500 mg/kg/day) and low pup viability during the 1st 4 days postpartum in rats (at a dose of 1500 mg/kg/day) indicate that there may be a risk of developmental mortality. Thus, the "Flow Chart C" is used to evaluate how much this risk may be to humans. Under "Signal Strength, Part A," a score of 1 is assigned since the signals were seen in both rats and rabbits and multiple effects were observed at several reproductive stages (during gestation and lactation periods). The score for the "Signal Strength, Part B" is -1 since the effects were associated with maternal toxicities without a dose-response relationship. The TI value for the effects seen in rabbits is estimated at ~ 13 (an estimated TD_{10} at 200 mg/kg/day and an ED_{90} at 16.7 mg/kg/day). Thus, the overall score for "Pharmacodynamics" is 0. The scores for "Concordance Between the Test Species and Humans," and "Class Alert" were 1 and 0, respectively, as explained above. The score for "Relative Exposure" remains -1 because the systemic exposures at 500 mg/kg/day in rabbits and 1500 mg/kg/day are expected to be >25 fold human exposure. The overall score

suggest accumulation of drug at this organ. The inflammation was graded as slightly higher than minimal severity.

Adult vs. juvenile: There were no differences in the toxicity profile between the adults and weaned/unweaned juvenile rats. The pharmacokinetic profiles between the weaned (age of 3-7 weeks old) and adult rats were more similar than that of the unweaned juvenile rats. The unweaned juvenile rats (age of 4-21 days) did not hydrolyze the prodrug efficiently at first. The systemic exposure to prodrug after a single dose of Ro 64-0796 was higher than that to the active metabolite and was estimated to be more than 10-fold higher than that in the adult rats. It is possible that the high systemic prodrug exposure was the cause to 75% mortality rate in the unweaned juvenile rats dosed with 1000 mg/kg/day Ro 64-0796. At this dose, only mild toxicities were detected following 6 months of repeated drug administration in adult rats. The hyrolysis of prodrug to its active metabolite increased following 2 weeks of repeated drug administration to the unweaned rats. However, it is unclear from the data whether the difference was associated with weaning or age. The prodrug to active metabolite ratio was similar in weaned and adults rats.

In conclusion, Ro 64-0796 and its active metabolite, Ro 64-0802, cause mild toxicities at high dosages and systemic exposures. The clinical dosage for the treatment of influenza viral infection is 75 mg b.i.d. for 5 days. The main toxicities caused by long-term (6-9 months) repeated administration of Ro 64-0796 were those related to kidneys and occurred at doses where systemic exposures to prodrug and active metabolites were more than 200- and 30-fold of those seen in humans. These results suggest a large safety margin and high therapeutic index.

The irritation to the GI system will probably be the dose-limiting toxicity in clinical usage, although the study in marmosets suggested that dividing up the doses may alleviate some of the GI irritation. The toxicokinetic data suggested that many of the toxicities may be associated with higher prodrug exposure. Human hydrolyzes the prodrug to its active metabolite at 7-fold greater than the rodents, suggesting an added safety margin. However, in severely hepatic impaired and very young pediatric (e.g., newborn) patients, special caution may be needed.

Addendum list:

1. Histopathology inventory.
2. Studies reviewed under IND.

ADDENDUM 1: Histopathology Inventory for NDA # 21087.ori

Study	W-142696	W-142761	W-138531 W-138539	W-143030	W-143066	W-143067	W-142938	W-142646	W-142697	W-143011	W-142940
Species	SD rats	SD rats	CDBR rats	SD rats	SD rats	SD rats	SD rats	Marmosets	Marmosets	Marmosets	Marmosets
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X	X		X	X	X	X
Bone Marrow smear	X	X	X	X (femur)	X	X		X	X (femur)	X (femur)	X
Bone	X(sternum)	X(femur)	X(sternum)					X	X (femur)	X	
Brain	X*	X*	X*	X	X*	X*		X*	X*	X*	X*
Cecum	X	X	X	X	X	X		X	X	X	X
Cervix	X		X								
Colon	X	X	X	X	X	X		X	X	X	X
Duodenum	X	X	X	X	X	X		X	X	X	X
Epididymis	X	X*	X	X	X	X	X*	X*	X	X*	X
Esophagus	X	X	X	X	X	X		X	X	X	X
Eye	X	X	X	X	X	X		X	X	X	X
Fallopian tube											
Gall bladder								X	X	X	X
Gross lesions	X	X	X				X	X	X	X	
Harderian gland	X		X								
Heart	X*	X*	X*	X*	X*	X*	X*		X*	X*	X*
Hyphophysis											
Ileum	X	X	X	X	X	X		X	X	X	X
Injection site							X				X
Jejunum	X	X	X	X	X	X		X	X	X	X
Kidneys	X*	X*	X*	X* (right)	X* (right)	X* (right)	X*	X*	X*	X*	X*
Lachrymal gland	X		X								
Larynx											
[illegible]er	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
[illegible]s	X*	X*	X*	X*	X*	X*	X*	X*	X	X*	X*
[illegible]ph nodes, cervical											
Lymph nodes mandibular	X		X								
Lymph nodes, mesenteric	X	X	X	X	X	X		X	X	X	X
Mammary Gland	X	X	X	X	X	X		X	X	X	
Nasal cavity											
Optic nerves	X										
Ovaries	X*	X*	X	X	X*	X*		X*	X*	X*	
Pancreas	X	X	X	X	X*	X*		X	X	X	X
Parathyroid	X*	X*	X	X	X		X	X	X*	X	X*
Peripheral nerve											
Pharynx											
Pituitary	X*	X*	X	X	X*	X*		X*	X*	X*	X
Prostate	X*	X	X	X	X*	X*		X	X	X	X
Rectum	X		X						X		
Salivary gland, submandibular	X	X	X	X	X	X		X	X	X	X
Sciatic nerve	X	X	X						X		
Seminal vesicles	X	X	X	X	X	X	X	X	X	X	X*
Skeletal muscle	X		X						X		
Skin	X	X	X	X	X	X		X	X	X	
Spinal cord	X	X	X	X				X	X	X	
Spleen	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Sternum									X		
Stomach	X	X	X	X	X	X	X	X	X	X	X*
Testes	X*	X*	X*	X	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X	X	X*	X*	X*	X*	X	X*	X*
Thyroid	X*	X*	X	X	X	X	X	X*	X*	X*	X*
Tongue	X	X	X	X	X	X		X	X	X	X
Trachea	X	X	X	X	X	X		X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X	X	X	X
[illegible]erus	X*	X	X	X	X*	X*		X	X	X	
[illegible]na	X		X								
[illegible]bal gland											
Axillary nerve				X	X	X		X		X	X
Lymph nodes, submandibular		X		X	X	X		X		X	X

* organ weight obtained

ADDENDUM 2: Studies Reviewed Under IND

Nonclinical Toxicology Review:

1. Ro 64-0796/002: A 4-week oral (gavage administration) range-finding toxicity study in the mouse (Study # 276/92-D6154; Report # W-142980; [redacted] Lot # 80202143; GLP; With QA-report; Study dates 4/21/98-5/21/98; Vol. 1, pp. 1-351).

Species/Strain: CD-1 mice	Route: Oral gavage	Duration of Dosing: 28 days
Weight Range on Day 1: M = 28-39 g; F = 22-29g	Age on Day 1: 6 weeks old	Dose Volume: 10 ml/kg

Data collected	Frequency/Occasion	Data collected	Frequency/Occasion
Clinical observation	Daily	Organ weights	Day 29
Body weight	Weekly	Histopathology	Day 29 on control & high dose groups only
Food consumption	Weekly		
Clinical pathology	Week 4	Toxicokinetics	During week 4 at 0.5, 1, 2, 4, 8, 12, 24 hrs postdose
Urinalysis	Week 3		

Important findings

Sex	Males						Females					
Dosage (mg/kg/day)	0	50	250	500	1000	1500	0	50	250	500	1000	1500
Number of animals:												
Main	12	12	12	12	12	12	12	12	12	12	12	12
Toxicokinetic[#]	6	21	21	21	21	21	6	21	21	21	21	21
T_{max} (hr)	-	0.5	0.5	1.0	1.0	4.0	-	0.5	0.5	1.0	1.0	8.0
C_{max} (µg/ml)	-	14.3	40.3	48.5	82.3	119	-	13.4	54.3	63.8	79.1	176
$AUC_{0\rightarrow 24h}$[†] (µg-h/ml)	-	25.3	120	211	567	946	-	13.1	94.5	192	436	748
Multiples human exp.	-	9	44	78	210	350	-	5	35	71	162	338
Number of deaths:	0	0	5	0	2	1	1	0	1	0	1	2
Cause: Unknown	0	0	3	0	0	0	0	0	0	0	1	0
Accident	0	0	1	0	2	1	1	0	1	0	0	1
Skin lesion	0	0	1	0	0	0	0	0	0	0	0	0
Renal lesion	0	0	0	0	0	0	0	0	0	0	0	1
Hematology												
Hemoglobin (g/dl)	14.9	15.0	15.3	14.9	15.1	16*	15.4	15.4	15.5	15.0	15.1	17.0
RBC (10^6/cmm)	9.3	9.6	9.6	9.6	9.46	10***	9.2	9.5	9.5	9.4	9.31	10.2
PCV (%)	45.0	46.2	46.4	45.1	45.5	49**	45.4	46.3	46.7	46.0	44.3	49.3
WBC (10^6/cmm)	4.7	4.6	4.1	3.9	3.2	2.8	4.9	4.0	4.9	2.6	3.2	5.4
Clinical chemistry												
ALK PHOS (IU/l)	122	204	147	164	170	333***	166	233	193	198	171	310***
Sodium (mmol/l)	145	146	149	147	144	137***	138	142	142	144	143	139
Chloride (mmol/l)	106	106	108	107	105	94***	99	102	104*	105**	103	103
Glucose (mmol/l)	3.3	4.1	6.4	5.5	5.7	8.1***	1.8	3.8**	3.7**	5.0***	3.7**	6.2***
Cholesterol (mmol/l)	3.7	4.0	3.1	3.6	4.2	5.3**	2.4	2.9	2.8	2.6	2.9	3.1*
Trigs. (mmol/l)	0.87	1.07	0.39	0.52	0.39	0.74	0.52	0.58	0.63	0.44	0.45	0.36
Histopathology:												
Kidney –												
Focal nephropathy												
Total # affected	2	3	1	2	1	7	2	3	2	2	1	5
Average grade	1.0	1.0	1.0	1.0	2.0	1.3	1.0	1.0	1.0	1.5	2.0	1.4
Spleen –												
Hemopoiesis												
Total # affected	12	-	-	-	-	11	11	1	-	-	-	10
Average grade	1.8	-	-	-	-	2.5	1.7	3.0	-	-	-	2.1