= Blood samples collected from 2-3 mice/sex/group † = Systemic exposure for the active metabolite Ro 64-0802 * P<0.05 ** P<0.01 *** P<0.001 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.

Treatment-related effects were mainly associated with the 1500 mg/kg dose group and consisted of renal and splenic toxicities. The mild but statistically significant increases in red blood cell counts, hemoglobin concentration, packed cell volume, and dose-related decreases in the white cell count in males may be an hematological manifestation of the slight to moderate splenic hemopoiesis in the high dose males. Although this histological finding was present in the high dose females, the changes in the same hematological parameters did not reach statistical significance. The minimal to slight focal nephropathy in the high dose probably caused most of the changes in the clinical chemistry parameters. The renal toxicities, while mild, may be dose-limiting in the longer-term study. Thus, a lower dosage may be considered for the 3-month range-finding study.

The toxicokinetic data clearly demonstrated that systemic exposure to the active metabolite, R64-0802, at all doses exceeded that in the clinical trials. Assuming that systemic drug exposure does not change following prolonged drug administration (which it is not expected to since Ro 64-0796 or its main and active metabolite, Ro 64-0802, does not cause autoinduction of its own metabolism), doses of 250 mg/kg/day will be sufficient for the carcinogenicity study to satisfy the criterion for 25X multiples of human exposure.

Comment: A dose-related and statistically significant increase in serum glucose was seen in females. The significance of this is unclear. While it suggests pancreatic or hepatic toxicity, related histological changes were not seen in this study. It was not seen in the 3-month toxicity study in the same species (see below).

2. Ro 64-0796/002: 13 week oral (gavage administration) range-finding toxicity study in the mouse (Draft report; Study # 276/98-D6154; Lot # 80202143; GLP; Study dates 8/17/98-11/19/98; Vol. 2, pp. 1-272).

Species/Strain: CD-1 mice	Route: Oral gavage		Duration of Dosing: 13 weeks
Weight Range on Day 1: M = 26-35 g; F = 19-27g		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	Week 14
Body weight	Weekly	Histopathology	Week 14 on control & high
Food consumption	Weekly		dose groups only
Clinical pathology	Week 13	Toxicokinetics	During week 12 at 0.5, 1, 2,
Urinalysis	Week 3		4, 8, 12, 24 hrs postdose

Important findings											
Sex		**Males**					**Females**				
Dosage (mg/kg/day)		0	100	250	600	1000	0	100	250	600	1000
Number of animals:											
Main		12	12	12	12	12	12	12	12	12	12
Toxicokinetic#		6	21	21	21	21	6	21	21	21	21
Pro-drug	T_{max} (hr)	-	0.5	0.5	0.5	0.5	-	0.5	0.5	2.0	0.5
	C_{max} (µg/ml)	-	16.9	40.3	25.3	41.5	-	8.5	16.1	20.3	37.3
	AUC_{0-24h}† (µg-h/ml)	-	8.6	36.6	96.6	202	-	10.1	33.0	81.9	191
	Multiples human exp.	-	***78***	***333***	***878***	***1836***	-	***92***	***300***	***745***	***1736***
Metabolite	T_{max} (hr)	-	0.5	1.0	1.0	4.0	-	0.5	0.5	1.0	1.0
	C_{max} (µg/ml)	-	11.1	31.5	43.9	54.7	-	26.5	26.5	38.7	47.1
	AUC_{0-24h}† (µg-h/ml)	-	33.9	138	314	474	-	26.5	76.7	182	367
	Multiples human exp.	-	***13***	***51***	***116***	***176***	-	***10***	***28***	***67***	***136***
Number of deaths:		0	0	1	2	3	0	0	0	1	3
Cause: Unknown		0	0	1	2	2	0	0	0	1	0
Accident		0	0	0	0	0	0	0	0	0	2
Urogenital lesion		0	0	0	0	1	0	0	0	0	0
Renal lesion		0	0	0	0	0	0	0	0	0	1
Hematology											
Hemoglobin (g/dl)		14.0	13.4	14.1	13.6	14.1	14.7	14.8	15.2	14.4	14.2
PCV (%)		45.6	43.8	46.3	44.5	45.2	47.1	46.1	47.8	45.7	44.8
Clinical chemistry											
ALK PHOS (IU/l)		91	92	139	113	163	137	178	218	192	173
Sodium (mmol/l)		150	146	148	147	146*	146	145	147	146	146
Potassium (mmol/l)		5.1	5.4	5.1	4.7	4.4	5.5	5.1	4.9	5.2	4.9
Chloride (mmol/l)		116	113	114	113	109*	114	113	114	113	114
Creatinine (µmol/l)		35	35	36	36	37	42	41	36	37	34*
Glucose (mmol/l)		5.9	8.2	6.6	8.4*	8.3*	8.4	7.1	6.7	8.9	8.2
Histopathology: *Kidney*											
Inflammatory foci											
Total # affected		4	4	4	5	1	3	2	6	6	5
Average grade		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Papillary mineralization											
Total # affected		3	5	3	6	9	4	6	3	3	3
Average grade		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Focal nephropathy											
Total # affected		3	3	3	5	8	3	3	7	5	4
Average grade		1.0	1.0	1.0	1.0	1.5	1.7	1.0	1.1	1.2	1.0
Tubular necrosis											
Total # affected		0	0	0	0	0	0	0	0	0	2
Average grade		0	0	0	0	0	0	0	0	0	1.5
Papillitis											
Total # affected		0	0	0	0	0	0	0	0	0	1
Average grade		0	0	0	0	0	0	0	0	0	1.0
Papillary necrosis											
Total # affected		0	0	0	0	1	0	0	0	0	1
Average grade		0	0	0	0	1.0	0	0	0	0	2.0

= Blood samples collected from 2-3 mice/sex/group

† = Systemic exposure for the active metabolite Ro 64-0802

* P<0.05

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.

There were a total of 10 deaths in both the main study and satellite groups. Only the one death caused by renal lesions in the high dose female group was considered to be related to the drug treatment. Body weight for high dose males dropped in week 13 only. Noisy respiration was noted in 3 high dose males at the end of week 1. There were various changes in the hematological and clinical chemistry parameters, some of which reached statistical significance either as compared to the control or by a dose response test. Most of the changes were slight. It is interesting that, this time, plasma glucose level elevated to statistical significance in the males but not females, as observed in the 1-month study. Treatment-related histopathological findings were limited to kidneys. These findings were slight in nature and non-dose-related. However, a death in the high dose female group was due to renal lesions. Thus, the high dose, 1000 mg/kg/day may be too high for the 2-year carcinogenicity study in this species.

The toxicokinetic data indicated that at 250 mg/kg/day, the systemic exposures to the pro-drug, Ro 64-0796, and the active metabolite, Ro 64-0802, both exceeded the 25X multiples of human exposure criterion. However, since there was a ~20% decrease on systemic exposures to the active metabolite over time (compare 4-week and 13-week AUC values) for both males and females, a dose above 250 mg/kg/day should be used in the carcinogenicity study.

Comment: The results for the toxicokinetic satellite groups were faxed on 5/24/99 to the Division.

CONCLUSIONS:

The two supporting range-finding studies for the 2 year mouse carcinogenicity study indicated that Ro 64-0796 is generally non-toxic. Even at the 1000 mg/kg/day, which gives over a 100-fold expected human exposure, only slight toxicities were observed with one death out of a total of 66 animals that may be related to drug treatment. Thus, the toxicity profile of this drug allows the sponsor to choose 25X multiples of human exposure as a criterion to select doses for the mouse carcinogenicity study. A high dose between 250 and 600 mg/kg/day will be sufficient to satisfy this criterion.

There is no regulatory action associated with this review. The review and the subsequent decision by the Exec CAC regarding the mouse carcinogenicity protocol will be included in the review associated with submission IND [redacted]

APPEARS THIS WAY
ON ORIGINAL

REPRODUCTIVE TOXICOLOGY

Summary:

A. Segment I – Fertility:

A1. Ro 64-0796 (also known as GS 4104): Oral (gavage) rat fertility and early embryonic development study (Report # W-142789; Study '60/R; Lot # 4104-02-F-1; GLP; With QA report; Study dates 6/6/97-8/15/97; Vol. 44, pp.78-238).

B. Segment II – Teratogenicity

B1. Ro 64-0796 (also known as GS 4104): Oral (gavage) rat development toxicity dose ranging study (Report # W-142777; Study 58/R; Lot # 4104-02-D-1; GLP; With QA report; Study dates 5/2/97-5/24/97; Vol. 45).

B2. Ro 64-0796 (also known as GS 4104): Oral (gavage) rat developmental toxicity study (Report # W-142791; Study 59/R; Lot # 4104-02-E-3; GLP; With QA report; Study dates 6/6/97-9/15/97; Vol. 46).

B3. Ro 64-0796 (also known as 4104): Oral (gavage) rabbit tolerance study (Report # W-142792; Study 62/R; Lot #'s 4104-02-D-1 & 4104-02-B-4; GLP; With QA report; Study dates 5/9/97-5/28/97; Vol. 47).

B4. Ro 64-0796 (also known as 4104): Oral (gavage) rabbit developmental toxicity dose-ranging study (Report # W-142782; Study 63/R; , Lot # 4104-02-E-2; GLP; With QA report; Study dates 5/30/97-6/25/97; Vol. 48).

B5. Ro 64-0796 (also known as 4104): Oral (gavage) rabbit developmental toxicity study (Report # W-142793; Study /64/R; , Lot # 4104-02-E-3; GLP; With QA report; Study dates 6/25/97-7/24/97; Vol. 49).

C. Segment III – Peri-/Post-Natal Toxicity

C1. Ro 64-0796 (also known as 104): Oral (gavage) rat pre- and post-natal development toxicity study (Report # W-143020; Study 061/R; UK; Lot # GPM0176; GLP; With QA report; Study dates 10/24/97-2/17/98; Vols. 50-51).

C2. Ro 64-0796/002: Supplementary oral study for effects on pre- and postnatal development in the rat (Report # B-169298; Study # 031R98; Hoffman-La Roche Ltd., Basel Switzerland; Lot # GPM 0192; GLP; With QA report; Study dates 3/3/98-4/17/98; Vols. 52-53).

C3. Ro 64-0796 (also Known as 4104): Oral (gavage) rat investigatory pre- and post-natal developmental toxicity study (Report # RR W-143071; Study 073/98 Lot # 80302643; GLP; With QA report; Study dates 9/18/98-11/5/98; Vols. 54-55).

Study Reviews:

A1. Ro 64-0796 (also known as [redacted]4104): Oral (gavage) rat fertility and early embryonic development study (Report # W-142789; Study [redacted]760/R; Lot # 4104-02-F-1).

Species/Strain: Sprague-Dawley rats	**Route:** Oral (gavage)	**Vehicle:** Acetate buffer, pH 4.0		**Dose volume:** 10 ml/kg
Duration of dosing: M: 4-weeks pre-mating to ~ 4 weeks after confirmation of pregnancy F: 2 weeks pre-mating to day 13 of pregnancy				**Caesarian section:** Day 14 of pregnancy
Weight Range on Day 1: M = 301-325 g; F = 176-200 g		**Age at start if dosing:** M = ~ 9 weeks; F = ~ 7 weeks		
Data collected: Clinical observations, body weights, food consumption, estrous cycle monitoring, mating and fertility parameters, gross macroscopic pathology, male reproductive organ weights & histopathology, uterine examination, pregnancy status, number of corpora lutea, and number and distribution of implantation.				
Important findings – Males				
Dosage (mg/kg/day)	0	50	250	1500
Number of animals at start of dosing	20	20	20	20
Body weights gain: (Days 1-57) (g)	169	155	152*	135**
Relative organ weights: Testes (g)	0.71	0.73	0.78**	0.77*
* P< 0.05	** P<0.01			
Important findings – Females				
Dosage (mg/kg/day)	0	50	250	1500
Number of animals at start of dosing	20	20	20	20
Body weight gain: Pretreatment days 1-11	5	7	12*	12*
(g) Pregnancy days 0-13	67	62	63	56**
NOEL for F_0 general effects = 50 mg/kg/day for males and 250 for females **NOEL** for F_0 reproductive performance = 1500 mg/kg/day **NOEL** for F_0 uterine parameters = 1500 mg/kg/day				

Except for the decreases in the body weight gain for both males and females, there were no other treatment-related effects. The increases in the relative testes weights arose mainly from the lower body weight in the mid- and high dose males. The absolute testis weights amongst all dosing groups were similar. It is known that the testis weights remains constant despite body weight loss, thus the increased testis weights are not considered related to the drug treatment.

B1. Ro 64-0796 (also known as [redacted]4104): Oral (gavage) rat development toxicity dose ranging study (Report # W-142777; Study [redacted]758/R; Lot # 4104-02-D-1).

Species/Strain: Pregnant Sprague-Dawley rats	**Route:** Oral (gavage)	**Duration of dosing:** Gestation days 6 to 17
Vehicle: Acetate buffer, pH 4.0	**Dose volume:** 10 ml/kg	**Caesarian section:** Day 20 of pregnancy
Weight Range on Day 5 of pregnancy: 209-267 g	**Age on day 1of pregnancy:** 10-12 weeks old	
Data collected: Clinical observations, body weights, food consumption, gross pathology, number of corpora lutea, number and distribution of implantation sites, fetal weights and fetal sexes for live fetuses, external examination of fetuses, placental weights, and toxicokinetics (day 17 of pregnancy at 1, 4, 8, and 24 hours postdosing).		

Important findings				
Dosage (mg/kg/day)	0	50	250	1500
Number of animals: Main study	7	7	7	7
Toxicokinetic study	0	3	3	3
T_{max} (h)	-	1.0	4.0	8.0
C_{max} (µg/ml)	-	3.77	13.10	61.10
C_{min} (µg/ml)	-	0.008	0.091	6.84
AUC_{0-24h} (µg-hr/ml)	-	14.8	114	727
Body weight change (g): Days 6-9 of pregnancy	14	14	12	13
Food consumption (g/rat/day): Days 6-9 of pregnancy	26.5	27.8	24.5	23.4*
NOEL for F_0 = 1500 mg/kg/day NOEL for F_0 uterine parameters = 1500 mg/kg/day NOEL for F_1 malformation = 1500 mg/kg/day				

Except for slight but not statistically significant or dose-related decrease in body weight gain during pregnancy days 6-9, there were no treatment-related effects in any parameters determined. The measurement of Ro 64-0796 was precluded because of its rapid hydrolysis in rat plasma as in the non-pregnant rats. The systemic exposure at 1500 mg/kg/day to the active metabolite, Ro 64-0801, in the pregnant female rats was slightly higher (<20%) as compared to the nonpregnant female rats. However, since the difference was so small and the pharmacokinetics were also linear in the pregnant rats, it is concluded that there is no difference in the pharmacokinetics for Ro 64-0796 between pregnant and non-pregnant rats.

B2. Ro 64-0796 (also known as [redacted]4104): Oral (gavage) rat developmental toxicity study (Report # W-142791; Study [redacted]59/R; Lot # 4104-02-E-3).

Species/Strain: Pregnant Sprague-Dawley rats	Route: Oral (gavage)	Duration of dosing: Gestation days 6 to 17
Vehicle: Acetate buffer, pH 4.0	Dose volume: 10 ml/kg	Caesarian section: Day 20 of pregnancy
Weight Range on Day 5 of pregnancy: 190-287 g	Age on day 1of pregnancy:10-12 weeks old	
Data collected: Clinical observations, body weights, food consumption, gross pathology, number of corpora lutea, number and distribution of implantation sites, fetal weights and fetal sexes for live fetuses, external examination of fetuses, placental weights, and toxicokinetics (day 17 of pregnancy at 1, 4, 8, 12, and 24 hours postdosing).		

Important findings				
Dosage (mg/kg/day)	0	50	250	1500
Number of animals: Main study	22	22	22	22
Toxicokinetic study#	0	3	3	3
T_{max} (h)	-	1.0	4.0	8.0
C_{max} (µg/ml)	-	3.77	13.30	63.60
C_{min} (µg/ml)	-	0.0126	0.0789	6.13
AUC_{0-24h} (µg-hr/ml)	-	13.4	101	825
Body weight change (g): Days 6-18 of pregnancy	94	93	95	85*
Food consumption (g/rat/day): Days 6-18 of pregnancy	27	27	27	25***
Number of litters examined	22	20	21	21
Number of fetuses examined	147	141	137	138

Dosage (mg/kg/day)	0	50	250	1500
Number of animals:	22	22	22	22
Skeletal findings:				
Minor malformation:				
Incomplete ossification of one or more neural arch				
% fetuses affected	0	0	0.7	1.4
% litters affected	0	0	4.8	4.8
Unossified 2nd sternebra				
% fetuses affected	0	0	1.5	1.4
% litters affected	0	0	9.5	9.5
Incomplete ossification of 3rd sternebra				
% fetuses affected	0	0	2.2	5.1
% litters affected	0	0	14.3	19.0
#: One ml of plasma was collected generally from 2 rats/dose/time point except for the time points of 1 and 24 hours postdosing. For these 2 time points, the plasma drug concentrations were determined from 3 rats/dose.				
NOEL for F_0 = 1500 mg/kg/day NOEL for F_0 uterine parameters = 1500 mg/kg/day NOEL for F_1 malformation = 1500 mg/kg/day				

There were statistically significant decreases in the body weight gain and food consumption in the 1500 mg/kg/day dose group from pregnancy days 6-18. During the last 2 days of pregnancy, the food consumption and body weight changes exceeded those of the control and making the average values of these 2 parameters not significantly different from those of the control.

Increased incidences of incomplete or no ossification in sternum and vertebrate was associated with the 250 and 1500 mg/kg/day groups. These observations were considered as minor developmental toxicities. These defects can be considered either as skeletal effects or delay in growth.

B3. Ro 64-0796 (also known as 4104): Oral (gavage) rabbit tolerance study (Report # W-142792; Study 62/R; Lot #'s 4104-02-D-1 & 4104-02-B-4).

Species/Strain: New Zealand White rabbits	Route: Oral (gavage)	Duration of dosing: 10 days
Vehicle: Acetate buffer, pH 4.0	Weight Range: 3-4 kg	Age on day 1: 4 months old	Dose volume: 10 ml/kg
Data collected: Clinical observations, mortality, body weights, food consumption, and toxicokinetics (last day of dosing at 1, 2, 4, 8, and 24 hours postdosing).		
Important findings		

Dosage (mg/kg/day)		0	50	250	500	750	1500
Number of animals:		3	3	3	3	3	3
Ro 64-0796	T_{max} (h)	-	1.0	1.0	1.0	2.0	-
	C_{max} (µg/ml)	-	2.37	13.30	28.00	23.30	-
	C_{min} (µg/ml)	-	0.0131	0.119	0.453	0.886	-
	AUC_{0-24h} (µg-hr/ml)	-	3.97	22.5	101	222	-
Ro 64-0801	T_{max} (h)	-	1.0	1.0	2.0	2.0	-
	C_{max} (µg/ml)	-	7.74	37.70	67.90	63.60	-
	C_{min} (µg/ml)	-	0.0349	0.380	1.440	4.040	-
	AUC_{0-24h} (µg-hr/ml)	-	13.3	82.5	251	627	-
Death/moribundity		0	0	0	0	1	3

Dosage (mg/kg/day)	0	50	250	500	750	1500
Number of animals:	3	3	3	3	3	3
Clinical observations:						
Reduced fecal output	1	0	0	2	3	3
Inappetence	0	0	0	0	3	3
Weight loss	0	0	0	0	3	3
Body weight change (kg): Days 1-8#	0.13	0.18	0.15	-0.06	-0.49	-0.42
Food consumption (g/rabbit/day): Days 6-9 of pregnancy	171.3	192.5	204.2	105.4	5.0	0.3
#: The weight change for the 1500 mg/kg/day group was calculated from days 1-4 because of the deaths of 2 animals while the average weight on day 8 for the 750 mg/kg/day group was determined from 2 animals because of the death of 1 animal in this group.						
NOEL = 250 mg/kg/day						

The 3 animals in the 1500 mg/kg/day received only 3-4 doses because of the severe deteriorated clinical conditions, including hypoactivity, prostration, tremors, few feces, hunched posture, and salivation. Marked weight loss was noted after 1st dose. The clinical condition was so severe for 2 animals that they were sacrificed on day 4 of treatment. The animal that died on day 4 in the 750 mg/kg/day dose group also exhibited similar clinical signs prior to death. Typical necropsy findings in these animals included erosion, reddening, and ulceration of stomach linings and fluid-filled, distended intestines. Thus 500 mg/kg/day was considered the maximum tolerated dose.

B4: Ro 64-0796 (also known as 4104): Oral (gavage) rabbit developmental toxicity dose-ranging study (Report # W-142782; Study 63/R; Lot # 4104-02-E-2).

Species/Strain: Mated New Zealand White rabbits	Route: Oral (gavage)	Duration of dosing: Gestation days 6-18		
Vehicle: Acetate buffer, pH 4.0	Weight Range: 3-4 kg	Age on day 1: 4 months old	Dose volume: 10 ml/kg	
Data collected: Clinical observations, body weights, food consumption, gross pathology, number of corpora lutea, number and distribution of implantation sites, fetal weights and fetal sexes for live fetuses, external examination of fetuses, placental weights, and toxicokinetics (day 18 of pregnancy at 0.5, 1, 2, 4, 7, 12, and 24 hours postdosing).				
Important findings				
Dosage (mg/kg/day)		0	50	250	500
Number of animals: Main Study		6	6	6	6
TK study		0	2	2	2
Ro 64-0796	T_{max} (h)	-	0.5	0.5	1.3
	C_{max} (µg/ml)	-	3.64	16.90	26.20
	C_{min} (µg/ml)	-	0.0205	0.161	1.140
	AUC_{0-24h} (µg-hr/ml)	-	6.45	34.3	152
Ro 64-0801	T_{max} (h)	-	1.0	1.0	2.0
	C_{max} (µg/ml)	-	7.90	41.10	79.60
	C_{min} (µg/ml)	-	0.0739	0.386	4.230
	AUC_{0-24h} (µg-hr/ml)	-	18.5	103	531
Death/moribundity due to abortion		0	0	0	1
Clinical observations: (% incidence#)					
Reduced or no fecal output		6.7	7	7	18
Food consumption (g/rabbit/day): Days 6-18 of pregnancy		136	128	125	94*
Abortion		0	0	0	1
#: % of incidence = sum of (number of animals with the clinical observation X total number of days with the clinical observation) ÷ (total number of animals/dose X total number of observation days)					

NOEL = 500 mg/kg/day

Rabbits did not convert the prodrug to the active metabolite as efficiently as rats. The systemic drug exposure ratio for prodrug and the active metabolite was ~ 1:3 for all doses. Except for the reduced fecal output, one unscheduled sacrifice due to abortion, and reduced food consumption, there were no other effects associated with high dose group. Thus, clear sign of maternal toxicity was not apparent. The result was contrary to the results from the previous dose range-finding study.

B5. Ro 64-0796 (also known as [redacted]4104): Oral (gavage) rabbit developmental toxicity study (Report # W-142793; Study [redacted]/64/R; Lot # 4104-02-E-3).

Species/Strain: Mated New Zealand White rabbits			**Route**: Oral (gavage)		**Duration of dosing**: Gestation days 6-18	
Vehicle: Acetate buffer, pH 4.0		**Weight Range**: 3-4 kg	**Age on day 1**: 4 months old		**Dose volume**: 10 ml/kg	
Data collected: Clinical observations, body weights, food consumption, gross pathology, number of corpora lutea, number and distribution of implantation sites, fetal weights and fetal sexes for live fetuses, external examination of fetuses, placental weights, and toxicokinetics (days & 18 of pregnancy at 0.5, 1, 2, 4, 7, 12, and 24 hours postdosing).						
Important findings						
Dosage (mg/kg/day)			0	50	150	500
Number of animals: Main Study			23	23	23	23
TK study			0	3	3	3
Ro 64-0796 - Prodrug	T_{max} (h)	Gestation Day 6	-	0.5	0.5	1.0
		Gestation Day 18		0.5	0.5	1.25
	C_{max} (µg/ml)	Gestation Day 6	-	3.01	8.93	18.80
		Gestation Day 18		7.30	9.31	27.90
	C_{min} (µg/ml)	Gestation Day 6	-	0.0117	0.0409	0.245
		Gestation Day 18		0.0599	0.165	0.647
	AUC_{0-24h} (µg-hr/ml)	Gestation Day 6	-	4.97	18.3	70.6
		Gestation Day 18		9.62	20.2	109
Dosage (mg/kg/day)			0	50	150	500
Number of animals: Main Study			23	23	23	23
TK study			0	3	3	3
Ro 64-0801 - Active Metab.	T_{max} (h)	Gestation Day 6	-	1.0	1.0	2.0
		Gestation Day 18		1.0	1.0	2.0
	C_{max} (µg/ml)	Gestation Day 6	-	7.30	20.00	52.50
		Gestation Day 18		7.24	17.50	73.20
	C_{min} (µg/ml)	Gestation Day 6	-	0.0401	0.137	0.935
		Gestation Day 18		0.0991	0.351	2.600
	AUC_{0-24h} (µg-hr/ml)	Gestation Day 6	-	17.9	57.7	211
		Gestation Day 18		17.0	45.8	306
Death/moribundity			0	0	0	2
Prescheduled sacrifice due to abortion			0	0	0	5
Clinical observations: (% incidence[a])						
Reduced or no fecal output			3.3	3.6	8.9	16.1
Body weight change(kg): Gestation days 6-18			0.21	0.23	0.16*	-0.26***
Food consumption (g/rabbit/day): Gestation days 6-18			135	122	120	57***

Dosage (mg/kg/day)	0	50	150	500
Number of animals:	23	23	23	23
Uterine parameters of the dams:				
Number died killed/aborted	0	0	0	7
Number of females w/ implantation at scheduled kill	23	20	21	12
Number of live fetuses	182	167	187	92
Mean % post-implantation loss	7.9	4.8	6.6	14.1
Mean % male fetuses	54.0	44.3	51.2	62.6
Major abnormality:				
Fused sternum				
% fetuses affected	0	0	0	2.2
% litters affected	0	0	0	8.3
Minor abnormalities:				
Common carotid artery arising from innominate artery				
% fetuses affected	13.7	18.6	18.2	34.8
% litters affected	56.5	70.0	76.2	91.7
Distended abdomen				
% fetuses affected	1.6	0	0.5	2.2
% litters affected	8.7	0	4.8	16.7
Cornua bent to skull hyoid				
% fetuses affected	1.6	4.2	1.6	4.4
% litters affected	8.7	30.0	14.3	33.3
Fused sternum				
% fetuses affected	0	2.4	1.1	2.2
% litters affected	0	15.0	9.5	8.3
Asymmetric insertion to the entire pelvic girdle				
% fetuses affected	1.6	0.6	4.8	5.4
% litters affected	13	5.0	33.3	33.3
Variation:				
≤ 14 centra on caudal vertebra				
% fetuses affected	2.7	5.4	2.7	12.0
% litters affected	21.7	35.0	23.8	58.3
Extra 13th rib				
% fetuses affected	37.4	46.1	54.0	57.6
% litters affected	87	90.0	95.2	91.7
Vestigial 13th rib				
% fetuses affected	8.8	18.0	12.8	15.2
% litters affected	47.8	80.0	71.4	75.0
Incomplete ossification on phalange of forelimb				
% fetuses affected	23.1	13.8	20.9	38.0
% litters affected	52.2	40.0	71.4	75.0

#: % of incidence = sum of (number of animals with the clinical observation X total number of days with the clinical observation) ÷ (total number of animals/dose X total number of observation days)
* P<0.05 *** P<0.001

NOEL for F_0 = 50 mg/kg/day
NOEL for F_1 = 500 mg/kg/day

Clear signs of maternal toxicity were apparent at the 500 mg/kg/day group. Two of the dams in this group had to be sacrificed early because of severe clinical signs and 5 others had prescheduled sacrifice due to signs of abortion. The surviving dams lost weight and ate 50% less food as compared to the control during treatment period. They also had higher mean percentage of post-implantation loss and live male fetuses (though not statistically significant) as compared

to the control. These effects were signs of developmental mortality and will be analyzed using the integrated tool that appears on CDER's web site at www.fda.gov/cder/meeting/advcomm/repro-tox62499.htm to assess their risk to human reproduction.

There were a variety of defects detected in the developing fetuses. Most of the observations were an increased incidence of minor skeletal abnormalities and variants. The sponsor has argued that most of the incidence values were within the historical control values and were not considered real. However, considering these skeletal effects as a bone syndrome, coupled with the ossification problem in rats and mortalities associated with bone problem in marmosets, it suggested that Ro 64-0796 and its active metabolite, Ro 64-0802 may have effects on bone. They were subjected to the analysis using the same integrated tool mentioned above and should be included in the Label.

Slight dose-related increases in the incidences of common carotid artery arising from innominate artery and distended abdomen were also detected in the F_1 generation. These defects were considered to pose little concern to humans since (1) they were commonly detected in this species of rabbits and (2) the incidences fell within the historical control values. They will not be included in the label.

C1. Ro 64-0796 (also known as /4104): Oral (gavage) rat pre- and post-natal development toxicity study (Report # W-143020; Study 061/R; Lot # GPM0176).

Species/Strain: Time-mated SD rats	Route: Oral (gavage)	Dosing period: Gestation day 6 to lactation day 20	
Vehicle: Acetate buffer, pH 4.0	Weight range: 201-225 g	Age range: 8-10 weeks old	Dose volume: 10 ml/kg
Data collected: F_0 generation: Clinical observations, mortality, body weights, food consumption, parturition duration, observations for abnormalities of nesting or nursing behavior, and kidney weights. F_1 generation: Clinical observations, mortality, pup body weights, pup food consumption, litter size and sex. Pups were culled 4 days postpartum to 4/sex/litter and their development was assessed by time to ear and eye opening, static righting reflex on day 5 of age, startle response and pupillary reflex on day 21 of age. After weaning (21-22 days postpartum), 20 pups/sex/dose (roughly 1/sex/litter/dose) were evaluated for learning, memory retention, and re-learning potential by E-shaped swimming maze test on day 28 of age, examined ophthalmoscopically on day 35 of age, and assessed for auditory function with SD-Screening system, sexual development (balanopreputial separation for males on day 35 of age and vaginal perforation for females on day 28 of age), and reproductive capacity (6 weeks postweaning), and kidney weights.			
Important findings – F_0 generation			
Dosage (mg/kg/day)	0	50	250	1500
Number of mated females	25	25	25	25
Number of unscheduled deaths/sacrifices	0	0	0	9
Number of litters produced	24	23	23	16
Clinical signs (% animals affected)				
Postdosing salivation	0	0	0	96
Difficult to dose	0	0	36	100
Internal "mass-like" area	0	0	0	72
Noisy or slow breathing	0	0	8	36
Hypoactive	0	0	0	40
Convulsion	0	0	0	16
Maternal body weight gain: Gestation days 6-17	80.5	79.0	81.5	71.0*
Maternal food consumption: Gestation days 6-9	24	25	25	20**
Relative kidney weights (% body weights):	0.83	0.86	0.86	0.95***

Natural delivery and litter data:				
Duration of parturition (min.)	125.2	125.7	153.1	181.7*
Live birth index	97.6	96.1	96.2	93.5
Preculling pup viability index	98.7	98.3	99.6	86.0*
Pup weight/litter on lactation day 21 (M/F)	~~43.3/42.0~~	~~42.9/40.9~~	~~45.7/44.1~~	~~39.2*/39.4~~
Important findings – F_1 generation				
Dosage (mg/kg/day)	0	50	250	1500
Number of animals examined & tested/sex	20	20	20	20
Body weight gain:				
Males (Weeks 1-11 postweaning)	418	395	399	372**
Females (Weeks 1-6 postweaning)	157	147	155	160
* P≤0.05 ** P≤0.01 *** P≤0.001				
NOEL for F_0 general effects = 50 mg/kg/day				
NOEL for F_0 reproductive performance = 50 mg/kg/day				
NOEL for F_0 uterine parameters = 1500 mg/kg/day				
NOEL for F_1 generation = 1500 mg/kg/day				

The maximum tolerated dose had probably been exceeded in this study. Nine dams were found dead or sacrificed *in extremis* in the 1500 mg/kg/day group in gestation day 21 or 22. Most to all of the animals in this group were difficult to dose, exhibited postdosing salivation, and had an internal "mass-like" area, which was believed to be, distended intestines. However, their body weight gain and food consumption was marginally affected. Statistical significant differences were observed only during part of the dosing period. The kidney weights were statistically significantly elevated. It took this group of dams longer to deliver their pups. Pups born to this group of dams also had a lower survival rate and the males had lower body weight gain during lactation. They may be related to the generally poor physical conditions observed in the dams. The surviving dams in the high dose group were also adverse to nurse. The observed effects can be attributed to maternal toxicities.

Parturition time may be one of the parameter adversely affected. Although it only reached statistical significance at the high dose (~45% longer than the control), the increase in the mean parturition time was dose-dependent (~ 22% longer for 250 mg/kg/day group as compared to controls). This parameter will be assessed with the integration tool to determine whether it poses great concern to human reproductive risk.

C2. Ro 64-0796/002: Supplementary oral study for effects on pre- and postnatal development in the rat (Report # B-169298; Study # 031R98; Lot # GPM 0192). In the previous study, pup weight and viability plus parturition time were adversely affected by the drug treatment. The present study was conducted to investigate the mechanism of action of these adverse effects by including clinical chemistry, urinalysis, and histopathology evaluation without post-weaning examinations.

Species/Strain: Time-mated Wistar rats	Route: Oral (gavage)	Dosing period: Gestation day 6 to lactation day 20	
Vehicle: Acetate buffer, pH 4.0	Weight range: 178-252 g	Age range: 8-10 weeks old	Dose volume: 10 ml/kg

Data collected:

F_0 generation: Clinical observations, mortality, body weights, food consumption, clinical chemistry (from satellite groups only; Ca^{2+}, phosphate, Mg^{2+}, Na^+, K^+, & Cl^-), urinalysis (from satellite groups only), parturition duration, observations for abnormalities of nesting or nursing behavior, organ weights (adrenal, brain, heart, kidneys, liver, ovaries, spleen, & thymus), histopathology (kidneys on all animals, esophagus, liver and stomach on control and high dose groups), toxicokinetic determination (blood samples collected from 2 rats/dose/time point at 1, 4, 8, and 24 hours postdosing on gestation days 6 and 21 and lactation 21).

F_1 generation: Clinical observations, mortality, pup body weights, pup food consumption, litter size and sex, milk uptake on lactation days 2-5, physical development (hair growth on days 5, upper incisor eruption on day 10, ear opening on days 16, & eye opening on day 18), & functional development (auditory startle on days 16 & pupil contraction on day 21), kidney weights.

Important findings – F_0 generation

Group		1	2	3	4	5
Dosage (mg/kg/day)		0	50	250	1500	1500[a]
Number of mated females:	Main study	21	21	21	21	21
	TK study	8	8	8	8	8
T_{max} (h)	Gestation day 6	-	1	1	8	-
	Gestation day 21	-	1	1	1	-
	Lactation day 21	-	1	8	8	-
C_{max} (µg/ml)	Gestation day 6	-	4.47	23.10	47.20	-
	Gestation day 21	-	5.53	23.00	51.20	-
	Lactation day 21	-	4.55	22.40	68.60	-
C_{min}	Gestation day 6	-	0.0247	0.266	28.40	-
	Gestation day 21	-	0.0254	2.250	4.64	-
	Lactation day 21	-	0.0216	0.592	31.10	-
AUC_{0-24h}	Gestation day 6	-	11.4	212	855	-
	Gestation day 21	-	15.7	171	637	-
	Lactation day 21	-	12.2	186	1100	-
Number of unscheduled deaths/sacrifices		0	0	2	0	2
Clinical signs (Frequency/# animals affected)						
Gestation days 7-21						
Hypersalivation		0/0	0/0	163/20	264/21	220/21
Urination when handled for weighing/dosing		0/0	0/0	15/7	37/11	40/13
Lactation days 1-22						
Hypersalivation		0/0	0/0	178/10	205/12	0/0
Urination when handled for weighing/dosing		0/0	0/0	3/2	13/1	0/0
Complete litter loss		1/1	1/1	0/0	3/3	2/2
Maternal body weight gain (g): Gestation days 7-14		58	52	66	58	45[*]
Lactation days 1-22		-11	-5	-6	-1	8
Maternal food consumption (g/rat/day): Gestation days 7-14		22	22	21	17[***]	17[***]
Lactation days 1-22		44	44	43	37	39
Uterine parameters in dams and litter data:						
Number of females pregnant		17	22	17	17	18
Number of females with live fetuses at delivery		14	17	14	15	13
Number of live fetuses at delivery		113	136	107	90	103
Live birth index (%)		93.3	99.1	94.2	96.8	95.5
Mean number of pups/litter		8.1	8.0	7.6	6.0	7.9
Duration of parturition (min.)		137	153	151	205	145

Group		1	2	3	4	5
Dosage (mg/kg/day)		0	50	250	1500	1500#
Number of mated females: Main study		21	21	21	21	21
Urinalysis (from animals with litters):						
Sodium (mmol/hr)	Gestation day 6/7	0.019	0.009	0.016	0.052	0.077
	Gestation day 19/20	0.020	0.016	0.045	0.063	0.064
	Lactation day 21/22	0.061	0.050	0.126	0.071	0.051
Chloride (mmol/hr)	Gestation day 6/7	0.035	0.017	0.027	0.071	0.094
	Gestation day 19/20	0.021	0.022	0.058	0.088	0.086
	Lactation day 21/22	0.079	0.065	0.154	0.107	0.066
Magnesium (mmol/hr)	Gestation day 6/7	0.0056	0.0032	0.0053	0.0077	0.0080
	Gestation day 19/20	0.0070	0.0053	0.0073	0.0087	0.0086
	Lactation day 21/22	0.0091	0.0079	0.0152	0.0088	0.0087
Histopathological findings (from animals with litters):						
Kidneys: Tubular basophilia:	# affected	6	9	11	12	7
	Average severity	1.0	1.0	1.0	1.3	1.3
Tubular mineralization	# affected	11	9	11	11	8
	Average severity	1.3	1.3	1.2	1.6	1.3
Stomach: Glandular ectasia	# affected	3	0	2	9	5
	Average severity	1.0	0.0	1.0	1.3	1.0
Relative kidney weights (% body weights):		1.042	1.147*	1.210**	1.298**	1.168**
Important findings – F_1 generation						
Group		1	2	3	4	5
Dosage (mg/kg/day)		0	50	250	1500	1500#
Number of fetuses examined		61	87	74	90	49
Number of litters examined		9	13	10	12	8
Pup viability index:	Lactation day 4	87.6	98.5	93.5	64.4***	90.3
	Lactation day 22	85.8	94.9	92.5	63.3***	83.5
Clinical observation: Lactation days 1-4						
Poor general condition (frequency/litters)		0/0	0/0	1/1	7/50	2/1
Pups without milk (% pups/% litters)		0/0	0/0	0/0	26.7/41.7	0/0
Pup weight/litter on lactation day 4		8.4	7.7	7.7	7.1**	7.5
Body weight gain/litter from Lactation days 1-20		32.4	30.2	29.7	25.3*	30.4

#: This group of dams was treated with Ro 64-0796 from gestation days 6-19.
†: The values are expressed as % fetuses failed the test/% litters with fetuses failing the test
* P≤0.05 ** P≤0.01 *** P≤0.001

NOEL for F_0 general effects = 50 mg/kg/day
NOEL for F_0 reproductive performance = 50 mg/kg/day
NOEL for F_0 uterine parameters = 1500 mg/kg/day
NOEL for F_1 generation = 1500 mg/kg/day

One death each in the groups 1 and 5 was due to dosing error, while one in group 5 died of dyscosia due to blockage of a fetus/pup at cervix. This dam carried 19 fetuses, an exceptionally high number for Wistar rats. Another group 3 dams died during lactation from unknown reasons. None of these deaths were attributed to drug administration.

The exposure to prodrug and the active metabolite was slightly higher (less than 1 fold) than that in the non-pregnant rats. The toxicological doses and effects were similar to SD non-pregnant rats. There were no unexpected toxicities associated with this study. As expected, shorter exposure duration (group 5) produced less effect. Although the duration of parturition for group 4 was prolonged as compared to the control, the value did not reach statistical significance. However, there was an indication that less live fetuses were born per litter in the group 4 in

addition to a higher incidences of pups without milk in the stomach, lower viability during the first 4 days of lactation, lower pup weights and weigh gain, and a higher incidence of pups exhibiting poor general condition. The results suggested drug effects on the parturition (duration), neonatal growth (reduced pup weight), and mortality (lower viability during the first 4 days of lactation). Again the study result confirmed what was found with Sprague-Dawley rats. On the other hand, the results were not seen in the dams treated from gestation days 6-17.

C3. Ro 64-0796 (also Known as 4104): Oral (gavage) rat investigatory pre- and post-natal developmental toxicity study (Report # RR W-143071; Study /073/98; Lot # 80302643). In the previous 2 Segment III studies, extended parturition, low pup viability and body weights were associated with a dosage of 1500 mg/kg/day in 2 strains of rats. The confounding factor in the 1st study (with Sprague-Dawley rats) was the high mortality rate (9 out of 25 dams) 1 or 2 days before delivery. The major confounding factor in the 2nd study was the low pregnancy rate (59% vs. 100% in the 1st study) and small litter (mean of 7 pups vs. 13 pups in the 1st study). In addition, it was reported that the diet used in the 1st Segment III study has been associated with 10-60% incidence of total litter loss in the UK. Thus, the present study was designed to sort out these confounding factors. In the toxicokinetic satellite study, 0.1 mg of dichlorvos in acetonitril was added to the blood samples before plasma drug concentration determination to prevent hydrolysis of prodrug, Ro 64-0796, to the active metabolite, Ro 64-0802, *ex vivo*.

Species/Strain: Time-mated Sprague-Dawley rats			**Route**: Oral (gavage)			**Vehicle**: Acetate buffer, pH 4.0	
Weight range: 201-225 g			**Age range**: 8-10 weeks old			**Dose volume**: 10 ml/kg	
Dosing duration: Group 1 – Gestation day 6 to postpartum day 21; 10 dams also received a slow bolus (over 2 minutes) i.v. injection of 10% (w/v) calcium gluconate from gestation days 14-21 at dosing volume of 1 ml. Groups 2 & 3 – Gestation day 6 to postpartum day 21 Group 4 – Gestation days 6 to postpartum day 21; during gestation day 14 to 21, dosage was fixed and based on the body weight recorded on gestation day 14 Group 5 – Gestation day 6 to postpartum day 21; also received a slow bolus (over 2 minutes) i.v. injection of 10% (w/v) calcium gluconate from gestation days 14-21 at dosing volume of 1 ml. Group 6 – Gestation days 6-17							
Data collected: F_0 generation: Clinical observations, mortality, body weights, food consumption, clinical chemistry (gestation days 5, 15, & 21 and postpartum day 21 on creatinine, calcium, phosphate, magnesium, sodium, potassium, & chloride), urinalysis (overnight samples on gestation days 6 & 19 and postpartum day 19), parturition observations (time of onset and completion of parturition & gestation period), toxicokinetic determination (blood samples collected from 3 rats/dose/time point at 1, 4, 8, and 24 hours postdosing on gestation days 6 and 21 (gestation day 17 for group 6) and postpartum day 21). F_1 generation: litter size, sexes, clinical observations, mortalities, pup body weights, pup development (ear opening on day 4, eye opening on day 15, static righting reflex on day 5, startle response on day 15, and pupillary light reflex on day 21)							
Important findings – F_0 generation							
Group		1	2	3	4	5	6
Dosage (mg/kg/day)		0	500	1500	1500	1500	1500
Number of animals:	Main study	30	20	20	20	20	20
	Biochemistry/Urinalysis	10	10	10	10	10	10
	TK study	15	15	15	15	0	15
Number of unscheduled deaths/sacrifices		0	2	4	1	3	0

Group			1	2	3	4	5	6
Dosage (mg/kg/day)			0	500	1500	1500	1500	1500
Number of animals:		Main study	30	20	20	20	20	20
		Biochemistry/Urinalysis	10	10	10	10	10	10
		TK study	15	15	15	15	0	15
Maternal body weight gain (g):								
		Gestation days 6-17	82.8	82.3	73.5***	73.0***	76.8*	73.5***
		Postpartum days 1-21	11.2	14.6	26.4***	24.6***	21.8**	16.2
Maternal food consumption (g/rat/day):								
		Gestation days 6-17	26.9	27.8	24.3***	24.5**	24.2**	24.7**
		Postpartum days 1-14	48.1	46.9	46.2	44.6	52.1	50.1
Uterine parameters in dams and litter data:								
Pregnancy index (%)			98.2	91.1	95.6	88.9	93.3	95.6
Number of females with live fetuses at delivery			54	40	40	40	28	43
Number of live fetuses at delivery			678	463	493	490	368	512
Live birth index (%)			99.6	97.7	99.8	96.5	100	99.2
Mean number of pups/litter			12.6	11.6	12.3	12.3	13.1	11.9
Duration of parturition (min.)			120.1	111.9	145.9**	155.0**	170.2***	130.9
Ro 64-0796, prodrug	T_{max} (h)	Gestation day 6	-	1.0	1.0	1.0	-	1.0
		Gestation day 21#	-	1.0	4.0	4.0	-	1.0
		Postpartum day 21	-	1.0	1.0	4.0	-	-
	C_{max} (µg/ml)	Gestation day 6	-	9.47	15.00	15.50	-	15.80
		Gestation day 21#	-	11.90	29.90	40.90	-	32.50
		Postpartum day 21	-	8.91	20.50	16.00	-	-
	C_{min}	Gestation day 6	-	0.0139	5.96	5.75	-	2.97
		Gestation day 21#	-	0.0767	5.71	1.51	-	0.102
		Postpartum day 21	-	0.0290	0.967	0.306	-	-
	AUC_{0-24h}	Gestation day 6	-	74.0	193	194	-	190
		Gestation day 21#	-	104	422	342	-	317
		Postpartum day 21	-	59.6	175	142	-	-
Ro 64-0902, active metabolite	T_{max} (h)	Gestation day 6	-	4.0	8.0	8.0	-	8.0
		Gestation day 21#	-	4.0	8.0	4.0	-	1.0
		Postpartum day 21		8.0	4.0	4.0	-	-
	C_{max} (µg/ml)	Gestation day 6	-	21.20	38.90	35.00	-	38.10
		Gestation day 21#	-	20.00	80.40	128.0	-	51.70
		Postpartum day 21		47.60	76.30	47.20	-	-
	C_{min}	Gestation day 6	-	0.0777	16.90	18.00	-	8.580
		Gestation day 21#	-	0.439	13.60	6.650	-	0.488
		Postpartum day 21		0.178	7.15	2.970	-	-
	AUC_{0-24h}	Gestation day 6	-	186	630	572	-	524
		Gestation day 21#	-	214	1040	887	-	779
		Postpartum day 21		338	763	485	-	-

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Group		1	2	3	4	5	6
Dosage (mg/kg/day)		0	500	1500	1500	1500	1500
Number of animals:	Biochemistry/Urinalysis	10	10	10	10	10	10
Clinical biochemistry:							
Creatinine (mg/dl)	Gestation day 6/7	0.8	0.7	0.8	0.7*	0.8	0.7**
	Gestation day 20/21	0.9	0.9	0.8*	0.8	0.8	-
Magnesium (mg/dl)	Gestation day 6/7	1.85	1.82	1.87	1.85	1.87	1.85
	Gestation day 20/21	2.04	2.09	2.28*	2.07	2.20*	-
Phosphate (mg/dl)	Gestation day 6/7	5.9	7.3***	7.7***	7.8***	7.6***	8.0***
	Gestation day 20/21	4.1	4.8	6.3**	4.8	5.9*	-
	Postpartum day 20/21	3.0	3.7	4.9*	5.1*	4.0	-
Sodium (mg/dl)	Gestation day 6/7	140	139**	140	139**	139*	139*
	Gestation day 20/21	141	139*	139*	140***	136***	-
Potassium (mg/dl)	Gestation day 6/7	5.1	4.8*	4.7**	4.6***	4.7*	4.8*
	Gestation day 20/21	4.8	4.3*	4.1**	4.2**	4.0***	-
Chloride (mg/dl)	Gestation day 6/7	107	106	107	106	107	107
	Gestation day 20/21	106	104*	103**	105	99***	-
	Postpartum day 20/21	105	104	102*	103	103	-
Urinalysis (from animals with litters):							
Sodium (mg/dl)	Gestation day 6/7	208	144***	66***	87***	103***	98***
	Gestation day 20/21	184	125*	42***	73**	58***	-
	Postpartum day 20/21	316	261*	210**	259	226**	-
Potassium (mg/dl)	Gestation day 6/7	230	240	115***	134***	166**	177
	Gestation day 20/21	250	189*	59.2***	88.2***	75.3***	-
	Postpartum day 20/21	280	245	234	244	222*	-
Chloride (mg/dl)	Gestation day 6/7	225	160***	90***	107***	130***	129***
	Gestation day 20/21	235	185*	65***	98***	85***	-
	Postpartum day 20/21	369	323	265**	325	289*	0.0087
Creatinine (mg/dl)	Gestation day 6/7	117	125	65***	73**	94	83*
	Gestation day 20/21	157	109*	33***	48***	42***	-
	Postpartum day 20/21	123	113	92*	101	85**	-
Magnesium (mg/dl)	Gestation day 6/7	54.36	44.13	33.09	34.29*	43.92	45.36
	Gestation day 20/21	108.23	79.77**	22.53***	29.53***	29.58***	-
Calcium (mg/dl)	Gestation day 6/7	36.4	25.1	9.0***	10.2***	14.3***	11.8***
	Gestation day 20/21	131.1	52.4**	9.8***	12.7***	15.8***	-
	Postpartum day 20/21	182.3	143.0	80.4***	97.8**	98.3**	-
Phosphate (mg/dl)	Gestation day 6/7	27.4	64.1*	93.6***	91.2***	110.4***	123.6***
	Gestation day 20/21	14.0	31.3	39.5*	36.5	54.6*	-

Important findings – F_1 generation							
Group		1	2	3	4	5	6
Dosage (mg/kg/day)		0	500	1500	1500	1500	1500#
Number of fetuses examined		667	433	466	448	320	500
Number of litters examined		54	38	40	39	27	43
Pup viability index:	Postpartum day 4	99.1	96.2	94.6*	90.0**	91.2*	98.2
	Postpartum day 22	100	97.1	97.4	100	96.2	99.7
Clinical observation:							
Pups not fed (% litter affected)		48	40	55	62	52	49

#: For group 6, blood samples for toxicokinetic analysis were taken on gestation day 17, the last day of dosing.
†: The values are expressed as % fetuses failed the test/% litters with fetuses failing the test
* P≤0.05 ** P≤0.01 *** P≤0.001

NOEL for F_0 general effects = 50 mg/kg/day NOEL for F_0 reproductive performance = 50 mg/kg/day NOEL for F_0 uterine parameters = 1500 mg/kg/day NOEL for F_1 generation = 1500 mg/kg/day

The results of this study confirmed what were found in the previous 2 studies. Most of the mortalities were a result of dosing error. Maternal toxicities were manifested as reduced body weight gain, reduced food consumption, and plasma and urine electrolyte imbalance (renal toxicities) at the 1500 mg/kg/day dose groups. Reducing the dosing period (group 6) reduced some of the toxicities while co-administration with calcium gluconate did not alleviate the Ro 64-0796-related toxicities. Prolongation of parturition time was again noted in this study as well as increased in pup viability during the 1st 4 days postpartum. Termination of drug administration before the onset of parturition (group 6) seemed to alleviate this effect.

Summary and Evaluation:

Studies assessing the oral administration of Ro 64-0796 on the fertility and early embryonic development, developing fetuses, and pre-and postnatal development have been carried out. These studies employed adequate number of animals and doses, administered the drug via the clinically relevant route, and were conducted according to Good Laboratory Procedure. The ADME profiles for this drug in rats and rabbits are quite similar to the humans. However, both species do not hydrolyze the prodrug to its active metabolite as efficiently as compared to humans and primates. It was estimated that the ratios of plasma prodrug to active metabolite concentrations were 1:3, 1:3, 1:10, and 1:22 for rats, rabbits, marmosets, and humans. Pregnancy, did not alter the pharmacokinetics of this drug in rats.

Oral administration of Ro 64-0796 and its active metabolite, Ro 64-0802, affected several reproductive toxicological parameters. They included parturition (increased duration in rats), lactation (increased incidence of pup without milk in the stomach, increased number of dams failed to nurse the offspring, and increased pup mortality during the first 4 days postpartum in rats), developmental mortality (increased incidence of post-implantation loss and abortion in rabbits and reduced pup viability during the first 4 days postpartum in rats), skeletal /quality of the milk), dysmorphogenesis (increased incidences of incomplete ossification of neural arch, unossified 2nd sterna, and incomplete ossification of 3rd sternebra in rats; increased incidences of fused sternum, cornua bent to skull hyoid, asymmetric insertion to the entire pelvic girdle, < 14 centra on cauda vetebra, extra 13th rib, vestigial 13th rib, incomplete ossification of phalange of forelimb in rabbits), alteration to growth (reduced pup weights in rats). These effects were evaluated using the integrated tool mentioned previously for the positive reproductive study results (Wedge or Flow Chart C).

Parturition: In the 3 Segment III reproductive studies using both Wistar and Sprague-Dawley rats, prolongation of the duration of parturition were observed at low and mid doses, but only reached statistically significant level at a dose of 1500 mg/kg/day. The high dose was associated with reduce food intake and body weight gain during gestation and renal toxicities (changes in the plasma and urine electrolytes). Prolongation of parturition is not considered a rare event and was given an overall score of 0 for "Signal Strength, Part B." The effect was measured only in rats. Other parturition endpoints (e.g., duration of gestation and live birth index) were not

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

for "Developmental Mortality" is 0, suggesting that these effects pose low concern to human reproductive risk.

Dysmorphogenesis: Incidences of incomplete ossification of neural arch, unossified 2nd sternebra, and incomplete ossification of 3rd sternebra were increased starting at a dose ≥ 250 mg/kg/day in rats. Increased incidences of minor skeletal abnormalities (fused sternum, cornua bent to skull hyoid, and asymmetric insertion to the entire pelvic girdle) and variants (≤ 14 centra on caudal vertebra, extra 13th rib, vestigial 13th rib, and incomplete ossification on phalange of forelimb) were associated with doses ≥ 50. These abnormalities were commonly observed in Sprague-Dawley rats and New Zealand white rabbits. The incidence rates for each individual abnormality/variant at non-maternally toxic doses were within the historical control values of untreated animals in the testing facility. However, these findings should be considered together as a skeletal/bone syndrome and subjected to the assessment by the integrated tool used thus far. Thus, for "Signal Strength, Part A, the overall score is 1 since the answer to "cross-species concordance" and "multiplicity of effects" were both yes. The score for "Signal Strength, Part B" is also 1 since maternal toxicity cannot account for all of the observed effects, which occurred at a dose-related increase in incidence rate. The overall score for "Pharmacodynamics" category is expected to be 0 even though the TI for rabbits was <5. The scores for "Concordance Between the Test Species and Humans," and "Class Alert" remains to be 1 and 0, respectively. While the score for "Relative Exposure" category should be 1 since the systemic exposures at low dose in the Segment II rat and rabbit studies were less than 10X of human exposure. Hence the total score for the bone effects is 4, which is suggestive of **a significant degree of concern for human reproductive risk.**

Alterations to Growth: The only affected endpoint under this class of developmental toxicity was reduced pup weights observed in the Segment III reproductive toxicity studies in rats. The effect was associated with dose ≥ 1500 mg/kg/day where maternal toxicity was apparent. Thus, the assessment process and conclusion should be the same as that used for analyzing prolongation of parturition time, which had an overall score of 0. As stated before, this endpoint should pose low concern to human reproductive risk.

Labeling Recommendations:

The only developmental effects that should be included in the Label is the skeletal abnormalities/variants analyzed under "Dysmorphorgenesis." This would place Ro 64-0796 under Pregnancy Category C. However, skeletal defects such as these should not be considered debilitating to humans. When considered individually, the incidence rates for these skeletal defects at non-maternally toxic doses were well within the laboratory historical control values. Many of the findings were common defects associated with the test species. In addition, most of the skeletal variants involved delayed ossification process, which may be considered as signs of growth retardation. Thus, a Label stating the association of skeletal effects with the drug treatment within the context of historic control values would be appropriate.

GENETIC TOXICOLOGY

Summary:

1. Evaluation of Ro 64-0802 (GS-4071) for mutagenic activity in the Ames test (Report # B-165592; Study # 129M97; Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 1163.85.36; GLP; With QA report; Study dates 7/7/97-7/18/97; Vol. 56, pp. 1-33).
2. Ro 64-0796 (also known as GS-4104): Mutagenicity test on GS-4104 in an *in vivo* mouse micronucleus test with toxicokinetics (Report # RR W-142699; Study # 96-TOX-4104-006; [redacted] Lot #1132-5-14; GLP; With QA report; Study dates 11/19/96-12/3/96; Vol. 56, pp. 34-94).
3. Ro 64-0796 (also known as GS 4104): Mutagenicity test with GS-4104 in the *Salmonella-Escherichia*/mammalian-microsome reverse mutation assay (Report # W-142698; Study # 96-TOX-4104-005; [redacted] Lot # 1029-78; GLP; With QA report; Study dates 6/28/96-8/4/96; Vol. 56, pp. 95-131).
4. Ro 64-0802 (GS-4071): Mouse lymphoma cell mutation test (ML/TK) (Report # B-165541; Study # 145M97; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 1163-85-36; GLP; With QA report; Study dates 7/30/97-9/12/97; Vol. 56, pp. 132-167).
5. Ro 64-0796 (also known as GS-4104): Mutagenicity test on GS-4104 chromosomal aberrations in human whole blood lymphocytes with and without exogenous metabolic activation (Report # W-142700; Study # 96-TOX-4104-007; [redacted] Lot # 1132-5-14; GLP; With QA report; Study dates 11/20/96-12/18/96; Vol. 56, pp. 168-197).
6. Ro 64-0796/002 spiked with five potential impurities: Chromosome aberration test with human peripheral blood lymphocytes (Report # B-167868; Study # 252M98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot #'s 80702944 for Ro 64-0796/002, 80202B2196 for Ro 64-6661/000, 1214-75-33 for Ro 64-1634, 80302B2187 for Ro 64-7943/001, 1214-143-20 for Ro 64-0952, 1214-163-22 for Ro 64-0951; GLP; With QA report; Study dates 10/12/98-11/23/98; Vol. 59, pp. 232-259).
7. Evaluation of a batch of Ro 64-0796/002, spiked with potential impurities, for mutagenic activity in the Ames test (Report # B-169878; Study # 264M98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot #'s 80702944 for Ro 64-0796/002, 80202B2196 for Ro 64-6661/000, 1214-75-33 for Ro 64-1634, 80302B2187 for Ro 64-7943/001, 1214-143-20 for Ro 64-0952, 1214-163-22 for Ro 64-0951; GLP; With QA report; Study dates 10/19/98-10/22/98; Vol. 61, pp. 1-33).
8. Assessment of the genotoxic relevance of the potential impurity Ro 64-1637 in preparations of the neuraminidase inhibitor Ro 64-0796/002 (Report # B-169881; Study # 132M97; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 1336-33-33 for Ro 64-1637; non-GLP; Without QA report; Study dates 7/10/97-5/7/98; Vol. 60, pp. 152-195).
9. Mutagenicity evaluation of Ro 64-0792/000 (intermediate of synthesis) in the Ames test (Report # B-167770; Study # 22M98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 7110052161; GLP; With QA report; Study dates 1/27/98-2/19/98; Vol. 58, pp. 105-136).
10. Mutagenicity evaluation of Ro 64-0789/000 (intermediate of synthesis) in the Ames test (Report # B-167769; Study # 21M98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 71002240; GLP; With QA report; Study dates 1/27/98-2/19/98; Vol. 58, pp. 137-168).

11. Mutagenicity evaluation of Ro 64-0795/000 (intermediate of synthesis) in the Ames test (Report # B-167807; Study # 23M98; F. Hoffmann-La Roche Ltd., Basel, Switzerland; Lot # 7110052161; GLP; With QA report; Study dates 1/27/98-2/12/98; Vol. 58, pp. 72-104).

Reviews:

1. Evaluation of Ro 64-0802 (GS 4071) for mutagenic activity in the Ames test (Report # B-165592; Study No. 129M97; Lot # 1163.85.36). The mutagenic potential of the major and active metabolite, Ro 64-0802, was examined in the *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 with or without S9 activation. S9 mixture was prepared from albino male rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the next 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 1 μg/plate sodium azide, for strain TA97 was 1 μg/plate ICR 191, for strain TA98 was 0.5 μg/plate 2-nitrofluorene, and for strain TA102 was 0.4 μg/plate mitomycin C. Four μg/plate of 2-aminoanthracene were used as the positive control for all strains both in the presence and absence of S9 activation. Two independent tests with concentrations ranging from 0 to 5000 μg/plate were carried out. A positive result was defined as a reproducible, dose-related increase in the number of *his*$^+$ revertants. The increase had to be at least 2-fold for strains TA1535 and TA98 and a 1.5-fold elevation for strains TA97, TA100, and TA102 over the number of spontaneous revertants in negative control.

Concentrations as high as 5000 μg/plate of Ro 64-0802 did not cause any cell toxicity. Concentrations of Ro 64-0802 ranged from 50-5000 μg/plate did not cause an increase in revertants per plate for all strains tested. Thus, the active metabolite of Ro 64-796 was considered not mutagenic in the assay employed.

Comment: In p. 56-13, under "Bacterial strains," *Salmonella* strain TA1537 was mentioned as one of the strain used in the Ames test. However, it's clear from other parts of the study report that this strain was not tested. The sponsor should be careful in making sure that all information submitted is correct.

2. Ro 64-0796 (also known as [redacted]-4104): Mutagenicity test on [redacted]4104 in an *in vivo* mouse micronucleus test with toxicokinetics (Report # RR W-142699; Study # 96-TOX-4104-006; Lot # 1132-5-14). Five mice/sex/dose/time point were orally administered (by gavage) with a single dose of 0 (deionized water; negative control), 80 mg/kg cyclophosphamide (positive control), 500, 1000, or 2000 mg/kg Ro 64-0796. The animals in the treatment groups were sacrificed at 24, 48, or 72 hours after dosing. Those in the positive and negative control groups were sacrificed at 24 hours post dose. An additional 2 male mice/dose/time point was dosed with a single dose of 0 (2 animals only), 1000, or 2000 mg/kg Ro 64-0796 by oral gavage and blood collected for toxicokinetic determination at 0.25, 0.5, 1, 2, 4, 6, and 8 hours post dose. Bone marrow from the hind limb bones was obtained from the animals in the main study. At least one thousand cells per animal were counted to determine the polychromatic/normochromatic erythrocyte ratio. The frequency of micronucleated cells was expressed as percent micronucleated cells based on a total of one thousand PCE counted per animal. An analysis of variance on either transformed or rank transformed proportions of cells with micronuclei per animal was carried out. When the analysis of variance reached a statistical significant level ($p<0.05$), a Dunnett's t test was used to determine if a particular dose group was significantly

different from the negative control. Two criteria were considered for a positive response: a significant dose-related increase in the number of micronucleated PCE's and the detection of a statistical significant positive response for at least one dose level. However, it called for the judgment of the study director to determine whether a test is positive if only one of the criteria was met.

A statistical significant change was not observed for any dose levels and assay time point. Since the majority of the prodrug, Ro 64-0796, is converted to the major and active metabolite, Ro 64-0802, only the exposure to the active metabolite was determined. The $AUC_{0\text{-}8h}$ values for the active metabolite at 1000 and 2000 mg/kg doses were 213 and 330 µg.h/ml, respectively, suggesting adequate drug exposures in the treated animals. Thus, under the conditions tested, Ro 64-0796 and Ro 64-0802 were not considered genotoxic.

3. Ro 64-0796 (also known as)4104): Mutagenicity test with)4104 in the *Salmonella-Escherichia*/mammalian-microsome reverse mutation assay (Report # W-142698; Study # 96-TOX-4104-005; Lot # 1029-78). The mutagenic potential of the prodrug, Ro 64-0796 (HCL salt), was examined in the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and *Escherichia coli* strain WP2*uvr*A, with or without S9 activation. S9 homogenate was purchased from It was prepared from male Sprague-Dawley rats that received intraperitoneal injection of Aroclor™ 1254 at 500 mg/kg. In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 2 µg/plate sodium azide, for strain TA91537 was 2 µg/plate)191, for strain TA98 was 1 µg/plate 2-nitrofluorene, and for *E. coli* strain WP2*uvr*A was 1 µg/plate 4-nitroquinoline-N-oxide. 2.5 µg/plate of 2-aminoanthracene were used as the positive control for all strains in the presence S9 activation. A range-finding study with strains TA100 and WP2*uvr*A and a mutagenicity assay with all strains were carried at 6 concentrations ranging from 0 to 5000 µg/plate. A positive result was defined as a dose-related increase in the number of *his*$^+$ or *trp*$^+$ revertants. The increase had to be at least 2-fold for strains TA100, TA98, and WP2*uvr*A and a 3-fold for strains TA1535 and TA1537 over the number of spontaneous revertants in the negative control.

Concentrations as high as 5000 µg/plate of Ro 64-0796 did not cause any cell toxicity. Concentrations ranging from 100-5000 µg/plate did not cause an increase in revertants per plate for all strains tested. Thus, the prodrug, Ro 64-796, was considered not mutagenic in the assay employed.

4. Ro 64-0802)4071): Mouse lymphoma cell mutation test (ML/TK) (Report # B-165541; Study # 145M97; Lot # 1163-85-36). The mutagenic potential of the active metabolite, Ro 64-0802, was assessed by examining its ability to induce *tk* mutations in L5178Y mouse lymphoma *tk*$^+$/*tk*$^-$ cells with and without activation. S9 fraction was prepared from Fü-albino male rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the remaining 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). The positive controls were 0.2 µg/ml of 4-nitroquinoline-1-oxide and 2 µg/ml benzo[a]pyrene in the absence and presence, respectively, of S9 activation. Two independent tests were carried out. A cytotoxicity range-finding test was carried out. Concentrations up to 3000 µg/ml Ro-64-0802 did not adversely affect all parameters that reflect the growth and survivability of the cells and thus were used in the mutagenicity assays. A positive mutagenic response was defined as a reproducible, dose-related, and statistically significant (>1 concentration) increase in the number of *tk*–mutant clones.

Concentrations up to 3000 μg/ml Ro-64-0802 did not cause an increase in the number of *tk*–mutant clones. Thus, the active metabolite is not considered mutagenic under the conditions of this assay.

5. Ro 64-0796 (also known as) 4104): Mutagenicity test on 4104 chromosomal aberrations in human whole blood lymphocytes with and without exogenous metabolic activation (Report # W-142700; Study # 96-TOX-4104-007; Lot # 1132-5-14). The ability of the prodrug, Ro 64-0796, to induce chromosomal aberrations was evaluated in cultured whole blood human lymphocytes with and without metabolic activation. S9 homogenate was purchased from . It was prepared from male Sprague-Dawley rats that had been treated with Aroclor™ 1254 to induce the mixed function oxidase enzymes. The positive controls were mitomycin C and cyclophosphamide in the absence and presence, respectively, of S9 activation. Peripheral blood lymphocytes were isolated from the blood of a single donor. Percentage of cells with aberrations and percentage of cells with more than one aberration were determined. Statistical analytical methods included a Cochran-Armitage test for linear trend and Fisher's Exact test to compare the percentage of cells with aberrations between treated and negative control cells. A positive mutagenic response was defined as a significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations. Evidence for increasing amounts of damage with increasing dose would also be considered as a positive response.

Severe cell cycle delay (mitotic index reduced 90% as compared to vehicle control) was observed in cultures dosed with 1670 μg/ml in the absence of metabolic activation. Moderate cell cycle delay (mitotic index reduced 62% as compared to vehicle control) was associated with drug concentration of 5010 μg/ml with metabolic activation. At the concentrations up to 1500 and 4990 μg/ml in the absence and presence of metabolic activation, respectively, no significant increase in the number of cells with chromosomal aberration was observed. Thus, the prodrug is not considered mutagenic under the conditions of this assay.

6. Ro 64-0796/002 spiked with five potential impurities: Chromosome aberration test with human peripheral blood lymphocytes (Report # B-167868; Study # 252M98; Lot #'s 80702944 for Ro 64-0796/002, 80202B2196 for Ro 64-6661/000, 1214-75-33 for Ro 64-1634, 80302B2187 for Ro 64-7943/001, 1214-143-20 for Ro 64-0952, 1214-163-22 for Ro 64-0951). Ro 64-0796/002 was spiked with 5 contaminants at the following concentrations: Ro 64-6661/000 and Ro 64-0952 at 1%, Ro 64-1634 and Ro-7943/001 at 0.2%, and Ro 64-0951 at 0.5%. Ro 64-6661/000, Ro 64-0952, and Ro 64-0951 are the degradation products while Ro 64-07943/001 and Ro 64-1634 are the synthesis by-products of Ro 64-0796. The mixture was tested for its mutagenic potentials in human peripheral blood lymphocytes with and without S9 metabolic activation. S9 mixture was prepared from male albino rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the rest 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). Peripheral blood lymphocytes were isolated from the blood of a single donor and stimulated by 0.0325 mg/ml phytohemagglutinin-M to enter mitosis. The top concentration in which the analysis for chromosome aberration was set at the one that caused a 50% reduction in mitotic index as compared to the control or at 10 mM, the maximal recommended concentration. Where possible, 100 metaphase cells were scored to determine the percentages of cells with structural chromosome aberrations excluding gaps, with gaps only, and with numerical chromosome changes. The statistical method used was Fisher's Exact Test. A positive

genotoxic response was defined as a significant increase ($p<0.01$) of the percent of cell with a particular kind of structural aberration (as defined above) over the negative and historic controls.

Concentrations of Ro 64-0796 spiked with 5 potential impurities ranging from 262 to 1640 μg/ml in the presence of S9 activation and 105 to 656 μg/ml in the absence of S9 activation did not cause a significant increase of percent of cells with chromosome aberrations over controls. Thus, the 5 impurities set at the specified levels are not mutagenic under the conditions tested.

7. Evaluation of a batch of Ro 64-0796/002, spiked with potential impurities, for mutagenic activity in the Ames test (Report # B-169878; Study # 264M98; Lot #'s 80702944 for Ro 64-0796/002, 80202B2196 for Ro 64-6661/000, 1214-75-33 for Ro 64-1634, 80302B2187 for Ro 64-7943/001, 1214-143-20 for Ro 64-0952, 1214-163-22 for Ro 64-0951). Ro 64-0796/002 was spiked with 5 contaminants at following concentrations: Ro 64-6661/000 and Ro 64-0952 at 1%, Ro 64-1634 and Ro-7943/001 at 0.2%, and Ro 64-0951 at 0.5%. Ro 64-6661/000, Ro 64-0952, and Ro 64-0951 are the degradation products while Ro 64-07943/001 and Ro 64-1634 are the synthesis by-products of Ro 64-0796. The mixture was tested for its mutagenic potentials in *Salmonella* strains TA1535, TA97, TA98, TA100, and TA102 with and without S9 metabolic activation. S9 mixture was prepared from male albino rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the remaining 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 1 μg/plate sodium azide, for strain TA97 was 1 μg/plate [redacted]191, for strain TA98 was 0.5 μg/plate 2-nitrofluorene, and for strain TA102 was 0.4 μg/plate mitomycin C. Four μg/plate of 2-aminoanthracene were used as the positive control for all strains both in the presence and absence of S9 activation. Two independent tests with concentrations ranging from 0 to 5000 μg/plate were carried out. A positive result was defined as a reproducible, dose-related increase in the number of *his*$^+$ revertants. The increase had to be at least 2-fold for strains TA1535 and TA98 and a 1.5-fold elevation for strains TA97, TA100, and TA102 over the number of spontaneous revertants in the negative control.

Concentration as high as 5000 μg/plate of Ro 64-0796 spiked with 5 possible impurities did not cause any cell toxicity nor an increase in revertants per plate for all strains tested. Thus, Ro 64-796 spiked with the 5 possible contaminants at the specified levels was considered not mutagenic in the assay employed.

8. Assessment of the genotoxic relevance of the potential impurity Ro 64-1637 in preparations of the neuraminidase inhibitor Ro 64-0796/002 (Report # B-169881; Study # 132M97; Lot # 1336-33-33 for Ro 64-1637). Initial batches of Ro 64-0796/002 were tested positive in standard Ames test with strain TA 1535 in the absence metabolic activation. The impurity that caused the positive mutagenic response had subsequently been identified to be Ro 64-1637, a 2-azido derivative. It is formed by partial reduction of the bis-azide, Ro 64-2988, a specified impurity present in Ro 64-0795 which is an azido-acetamide and a direct synthetic precursor of Ro 64-0796. Two batches that contained 0.10 and 0.14% of the 2-azido impurity were reevaluated in the standard Ames test with strain TA1535 and were considered to be positive. The 2 batches with 0.03 and 0.04% of impurity increased the number of revertants less than the 2-fold and were considered not positive, and the one batch with undetectable impurity did not increased the number of revertants as compared to the negative control. Thus, the acceptable limit for this impurity was set at 0.01% for the CMC process.

To ensure that the mutagenic effects observed with impure batches of Ro 64-0796 was due solely to the 2-azido impurity (Ro 64-1637), this compound was synthesized and tested in strain TA1535. It caused a 2.5-fold increase in spontaneous mutation rate at 1 μg/plate and a 150-fold increase at 316 μg/plate. The results added another piece of evident to suggest that Ro 64-1637 was the cause of positive mutagenic response in the Ames test.

Subsequently, the sponsor performed another experiment to estimate the amount of azide (formed from the metabolism of Ro 64-1637) needed to induce the same number of revertants as that induced by 1 μg/plate of sodium azide. Then, a set amount of Ro 64-1673 was incubated with strain TA1535 under the same conditions as the Ames test. The amount of azide metabolized from Ro 64-1637 was then calculated and found to correlate well with the estimated number. These estimation and extrapolation, the sponsor claimed, further support the hypothesis that azide from the metabolism of Ro 64-1673 was the cause for the positive mutagenic response in the impure Ro 64-0796 batches.

Finally, the sponsor argues that since azide is catalyzed together with O-acetylserine by O-acetylserine(thiol) lyase to form azidoalanine which is further metabolized to an ultimate mutagen, it has little relevance to mammalian cells. This enzyme is found only in bacteria, plants, and other lower eukaryotes. In addition, azide is not carcinogenic in rats. Thus, it was felt that a small amount of 2-azido impurity should not present any safety concern.

9. Mutagenicity evaluation of Ro 64-0792/000 (intermediate of synthesis) in the Ames test (Report # B-167770; Study # 22M98; Lot # 7110052161). 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 mutagenic potential of Ro 64-0792/000 was tested in *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 with and without S9 metabolic activation. S9 mixture was prepared from male albino rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the remaining 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 1 μg/plate sodium azide, for strain TA97 was 1 μg/plate [redacted]191, for strain TA98 was 0.5 μg/plate 2-nitrofluorene, and for strain TA102 was 0.4 μg/plate mitomycin C. Four μg/plate of 2-aminoanthracene were used as the positive control for all strains both in the presence and absence of S9 activation. Two independent tests with concentrations ranging from 0 to 5000 μg/plate were carried out. A positive result was defined as a reproducible, dose-related increase in the number of *his*$^+$ revertants. The increase had to be at least 2-fold for strains TA1535 and TA98 and a 1.5-fold elevation for strains TA97, TA100, and TA102 over the number of spontaneous revertants in the negative control.

Precipitation of the test material was observed starting at 1666 μg/plate. Toxicity was observed starting at 500 μg/plate (in one of the experiment only). There was no increase in the mutant frequencies in any strains tested both with and without metabolic activation. Thus, Ro 64-0792 was considered not mutagenic under the conditions tested.

10. Mutagenicity evaluation of Ro 64-0789/000 (intermediate of synthesis) in the Ames test (Report # B-167769; Study # 21M98; Lot # 71002240). 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. Its mutagenic potential was

tested in *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 with and without S9 metabolic activation. S9 mixture was prepared from male albino rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the remaining 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 1 μg/plate sodium azide, for strain TA97 was 1 μg/plate ICR 191, for strain TA98 was 0.5 μg/plate 2-nitrofluorene, and for strain TA102 was 0.4 μg/plate mitomycin C. Four μg/plate of 2-aminoanthracene were used as the positive control for all strains both in the presence and absence of S9 activation. Two independent tests with concentrations ranging from 0 to 5000 μg/plate were carried out. A positive result was defined as a reproducible, dose-related increase in the number of *his*$^+$ revertants. The increase had to be at least 2-fold for strains TA1535 and TA98 and a 1.5-fold elevation for strains TA97, TA100, and TA102 over the number of spontaneous revertants in the negative control.

Slight precipitation of the test material was detected starting at 3333 μg/plate. At 5000 μg/plate, the strong precipitation necessitated the scoring of mutant colonies manually. There was no increase in the mutant frequencies in any strains tested both with and without metabolic activation. Thus, Ro 64-0789 was considered not mutagenic under the conditions tested.

11. Mutagenicity evaluation of Ro 64-0795/000 (intermediate of synthesis) in the Ames test (Report # B-167807; Study # 23M98; 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 impurity gives rise to a 2-azido impurity, Ro 64-1637, which was tested positive in *Salmonella typhimurium* strain TA1535. The mutagenic potential of Ro 64-0795/000 was tested in *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 with and without S9 metabolic activation. S9 mixture was prepared from male albino rats that received intraperitoneal injections of phenobarbital (a total of 4 injections at 30 mg/kg for the 1st day and 60 mg/kg for the remaining 3 days) and β-naphtholflavone (dissolved in corn oil at 80 mg/kg on the 3rd day). In the absence of S9 activation, the positive control for strains TA1535 and TA100 was 1 μg/plate sodium azide, for strain TA97 was 1 μg/plate [redacted] 191, for strain TA98 was 0.5 μg/plate 2-nitrofluorene, and for strain TA102 was 0.4 μg/plate mitomycin C. Four μg/plate of 2-aminoanthracene were used as the positive control for all strains both in the presence and absence of S9 activation. Two independent tests with concentrations ranging from 0 to 5000 μg/plate were carried out. A positive result was defined as a reproducible, dose-related increase in the number of *his*$^+$ revertants. The increase had to be at least 2 fold for strains TA1535 and TA98 and a 1.5-fold elevation for strains TA97, TA100, and TA102 over the number of spontaneous revertants in the negative control.

At 5000 μg/plate, the strong precipitation necessitated the scoring of mutant colonies manually. At this concentration, there was a reproducible, 2-3-fold increase in the mutant frequencies (as compared to the negative control) in strain TA1535 in the presence of S9 metabolic activation and a non-reproducible 2-fold increase in the mutant frequencies without S9 activation. No such increase was associated with any other strains. Thus, this batch of Ro 64-0795 was considered mutagenic which most likely is due to the 2-azido impurity, Ro 64-1637, arising from partial reduction of the specified impurity, Ro 64-2988.

Conclusion for Genetic Toxicology Section:

The neuraminidase inhibitor, Ro 64-0796 and its major and active metabolite, Ro 64-0801 have been tested in the standard battery of tests for genotoxicity, including the bacterial mutagenic tests, the *in vitro* mammalian mutagenicity tests, and the *in vivo* mouse micronucleus test. The results of these tests have indicated that both compounds are not considered genotoxic. Some of the degradation products and precursors in the synthetic pathway of Ro 64-0796 were also evaluated in the Ames test with 5 *Salmonella typhimurium* strains. All but one (Ro 64-0795, the direct synthetic precursor of Ro 64-0796) was considered negative in their mutagenic potential.

The batch of Ro 64-0795 tested contained a specified impurity Ro 64-2988, a bis-azide that can give rise to a 2-azido impurity, Ro 64-1637 through partial reduction. The 2-azido impurity has been detected in some of the earlier batches of Ro 64-0796 and tested positive in *Salmonella typhimurium* strain TA1535. Most of the evidence indicated that the positive mutagenic response was due to the presence of the Ro 64-1673 that is metabolized to produce azide and Ro 64-0796. Azide is a known bacterial mutagen. The acceptable limit for Ro 64-1673 has been set at 0.01% since at 0.03%, the mutagenic response as tested by the Ames method was considered negative.

Addendum list:

1. Studies reviewed under IND.

ADDENDUM 1: Study Reviewed Under IND

Study Review:

1. Mutagenicity test with [redacted]-4104-02 in the *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay (Report [redacted]96-TOX-4104-011; [redacted] ref. # 18132-0-409; 11/27/96-6/17/97). A number of batches of [redacted]-4104-02 was tested for their genotoxic potentials using the *Salmonella-Escherichia coli*/ mammalian-microsome reverse mutation assay since changes in the impurity profile and the salt form were observed for the new batches of Ro 64-0796/002 to be used for the clinical studies. These lots included 1116-12-30, 1116-101-11, 1116-101-30, 1177-27-04, 1116-102-20, 1116-103-17, 1116-104-19, 1132-5-14, 1132-5-14, 1136-79-27, 1136-80-28, 1174-135A, 4104-02-B-1, [redacted]-4104-02-B-1, [redacted]-4104-02-B-2, 4104-02-B-3, 4104-02-B-4, 4104-02-B-5, [redacted]-4104-02-C-1, 4104-02-D-1, 4104-02-D-2, 4104-02-E-2, 4104-02-E-3, 4104-02-F-1, 4104-02-F-2. In addition, Lot # 1029-92 for [redacted]-4104-01 and Lot # 1163-85-36 for [redacted]-4071 (the active metabolite) were also tested. Liver S9 homogenate was prepared from male Sprague-Dawley rats that had been injected with 500 mg/kg Aroclor™ 1254. Some of the lots were tested with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvr*A, but most of the lots were checked with *Salmonella typhimurium* strain TA1535 in the absence of S9 rat liver microsomal activation. The reason for it was presumably that under such conditions, the greatest fold-increase in revertant (hence mutagenicity) were observed. The following table shows the lots that have been tested positive in the strain and conditions assayed:

Table 2. Lots that were positive in the *Salmonella-Escherichia coli*/ mammalian-microsome reverse mutation assay under the conditions indicated.

Test Article Lot #	Positive in Tester Strain	+ S9 Activation*	- S9 Activation*
1132-5-14	TA1535§	8.1	11.0
4104-02-B-1	TA1535	3.5	9.6
1116-12-30	TA1535	-	3.5
1029-92	TA1535	5.7	8.4
1132-5-14	TA1535	5.3	6.8
[redacted]-4104-02-B-1	TA1535	NA	6.2
1177-27-04	TA1535	NA	13
1116-102-20	TA1535	NA	3.6
1116-104-19	TA1535	NA	3.3

* The number indicates maximum fold increase in the number of revertant as compared to the control at a concentration of 5 mg/plate.

§ This lot was tested with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvr*A; however, it was only positive in strain TA 1535.

NA = Not applicable, the test article was not tested under this activation condition.

- = No positive response observed.

From the results of this report, it seems that lots that were received by [redacted] after February, 1997 were not mutagenic in *Salmonella typhimurium* strain TA1535 in the absence of S9 activation. This fact suggested that the mutagenic impurities probably existed in the earlier lots and were subsequently cleaned up. This appeared to be the case. It is mentioned in the

report that the mutagenic impurity had been identified as a 2-azido adduct of Ro 64-0796/000 [redacted], an expected impurity since sodium azide was used for the drug synthesis. It may also explain why increases in revertants were seen only in strain TA1535, which gives a positive response to sodium azide and related mutagens. It is mentioned in the report that methods have been introduced in the manufacturing processes to eliminate the 2-azido adduct from the future lots. It is reassuring that the two lots, 4104-02-B-2 and 4104-02-B-3, to be used in the planned clinical studies have been tested negative in *Salmonella* strain TA1535. In addition, lot 4104-02-B-2 was also negative in all 5 bacteria strains tested. These two lots contained less than 0.02% of 2-azido adduct while lots that had >0.16% of this impurity were mutagenic to strain TA1535.

Comments: Lot #1132-5-14, which tested positive twice in the absence of S9 activation with *Salmonella typhimurium* strain TA1535, was found to be non-genotoxic in the chromosomal aberration assay in human whole blood lymphocytes with and without exogenous metabolic activation and in the *in vivo* mouse micronucleus assay.

Comments: Lot # 1029-92, tested to be mutagenic in this assay, was used in the one-month oral toxicity test in marmosets.

APPEARS THIS WAY
ON ORIGINAL

PHARMACOKINETICS/TOXICOKINETICS

D. Pharmacokinetic and Tissue Distribution in Pregnant Animals

Summary:

D1. Whole body phosphor imaging in the male and pregnant rat following single oral administration of [^{14}C] Ro 64-0796 (Report # W-142778; Study # DHB06001; [redacted] Lot #s 121-169-059 and 4104-02-B-2 for [^{14}C]-labeled and unlabeled Ro 64-0796, respectively; GLP; With QA report; Study dates 3/14/97-4/22/97; Vol. 68, pp. 123-226).

D2. The disposition and pharmacokinetics of Ro 64-0796 and Ro 64-0802 after single oral administration of the pro-drug, Ro 64-0796, to pregnant rabbits (Report # W-142986; Study # DHB07101; [redacted]; Lot #s 121-169-059, LC571 and 4104-02-E-3 for [^{14}C]-labeled and unlabeled Ro 64-0796, respectively; GLP; With QA report; Study dates 10/97-12/98; Vol. 70, pp. 1-122).

D3. An investigation into the secretion of drug-related material into milk after oral administration of 14-carbon labeled Ro 64-0796 to the rat (Report # W-143048; Study #'s 163193 and DHB11701; [redacted] non-GLP; Without QA report; Study dates 7/14/98-12/7/98; Vol. 70, pp. 123-237).

Reviews:

D1. Whole body phosphor imaging in the male and pregnant rat following single oral administration of [^{14}C] Ro 64-0796 (Report # W-142778; Study # DHB06001; Lot #s 121-169-059 and 4104-02-B-2 for [^{14}C]-labeled and unlabeled Ro 64-0796, respectively). Four male and pregnant females (day 16 of gestation) each were given a single oral dose of 20 mg/kg (free base) [^{14}C] Ro 64-0796. Tissue concentrations of radioactivity were quantified by whole body phosphor imaging using [redacted] at 2, 7.5, 24, and 72 hours post-dose from single animals. Triplicate measurements were made for each tissue.

The distribution pattern of [^{14}C] Ro 64-0796 in both male and pregnant female rats was similar. Radioactivity was rapidly and widely distributed throughout the tissues with maximum concentrations of radioactivity found in the content of the gastrointestinal tract initially. The highest levels of radioactivity in the majority of tissues of males and all the tissues of the pregnant females were detected at 2 hours postdose. The highest tissue radioactivity was associated with the excretory organs: liver, kidney cortex, and kidney medulla. Pregnant female rats had a higher systemic drug exposure than the males. In a high proportion of tissues, radioactivity concentrations were greater than in blood.

Placental transfer to the fetuses was low but quantifiable up to 7.5 hours post-dose. Higher radioactivity levels were measured in tissues associated with fetal development (yolk sac and placenta) up to 24 hours postdose.

Elimination from organs was rapid. Complete elimination in both males and pregnant females was achieved at 72 hours postdose with the exception of the pigmented areas of skin and eye and the organs of biotransformation. Detectable levels of radioactivity were also found in the contents of the gastrointestinal tract and adipose tissue of pregnant rats at this time point. Excretion by the fecal route was 42% of total radioactivity.

D2. The disposition and pharmacokinetics of Ro 64-0796 and Ro 64-0802 after single oral administration of the pro-drug, Ro 64-0796, to pregnant rabbits (Report # W-142986; Study # DHB07101; Lot #s 121-169-059, LC571 and 4104-02-E-3 for [^{14}C]-labeled and unlabeled Ro 64-0796, respectively). Nine pregnant rabbits received a single oral dose of 50 mg/kg [^{14}C] Ro 64-0796 (phosphate salt) on day 18 of gestation. One rabbit each was sacrificed at 0.5, 1, 2, 4, 7, 12, 24, and 72 hours postdose. Terminal blood, maternal samples, fetuses, bile and bladder urine were assayed for radioactivity. The metabolites were also characterized by

Absorption of Ro 64-0796 is high (>84% of administered dose) in rabbits and excretion is predominately via the renal route (84%).

Distribution was widespread and extensive. The radioactive concentrations in lungs were consistently higher than those in plasma at all time points measured. Placental transfer was limited. Fetal drug exposure was estimated to be 11-56% of the exposure in dams.

Metabolites were similar to those found in rats and mice including the hydroxylated derivatives (in the pentyl side-chain) of both Ro 64-0796 and Ro 64-0802. Glucuronides of Ro 64-0802, the active metabolite, accounted for ~ 10% of urinary metabolites.

D3. An investigation into the secretion of drug-related material into milk after oral administration of 14-carbon labeled Ro 64-0796 to the rat (Report # W-143048; Study #'s 163193 and DHB11701). Seven time-mated Sprague-Dawley rats received a single oral dose of 10 mg/kg [^{14}C]-labeled Ro 64-0796 (free base) on postpartum day 11. Milk (~ 100 µl) and maternal blood (~ 200 µl) were collected from 5 rats at 1, 4, 7, 12, 24, 48, and 72 hours postdose for measurement of radioactivity. Another group of 12 lactating rats received a single dose of 10 mg/kg unlabeled Ro 64-0796 (phosphate salt) on postpartum day 13. Milk and plasma were collected from 2 animals each at 0.5, 1, 3, 6, 12, or 24 hours postdose and concentrations measured by)Pharmacokinetic parameters of Ro 64-0796 and Ro 64-0802 in rat plasma and milk were calculated on pooled data using non-compartmental methods, because several of the time-points were missing from the 1st group of rats and only single data points were obtained from each rat in the 2nd group.

Both Ro 64-0796 and Ro 64-0802 were secreted into milk to a similar extent. Total drug exposures for both the prodrug and active metabolite in milk were 2-3 times those in plasma at a single dose of 10 mg/kg. The prodrug and active metabolite accounted for all of the administered drug material at early time point, other metabolites also circulated at later times.

Absorption:

Oral absorption of Ro 64-0796 is high in all species studied. It was at least 68% in rats, 72% in marmosets, 80% in man. Oral bioavailability for the active metabolite, Ro 64-0802, is low (< 5% in marmoset and rats) but can be increased ~ 10-fold in marmosets and 3-20 fold in rat if the prodrug, Ro 64-0796, was administered orally instead.

Distribution:

The binding of Ro 64-0796 to plasma proteins ranges from 16% in rabbits to 42% in man while the active metabolite is not protein bound in any species. Ro 64-0796 and its active metabolite are distributed rapidly and extensive in all tissues examined except for CNS. They are transferred freely through blood-placenta barrier and secreted into milk. There was evidence that the developing fetuses were exposed to Ro 64-0796 and its active metabolite.

Metabolism & Elimination:

The active metabolite, also the active neuraminidase inhibitor, Ro 64-0802, dose not appear to be metabolized by any species except for limited formation of glucuronides in rabbits. It is excreted exclusively via kidneys in humans unchanged after intravenous administration. In marmosets, renal excretion is the predominate route of elimination. Excretion in rats appeared to involve renal, fecal, and P-glycoprotein transporter in the gut-wall.

PK parameters:

The following table is adopted from the one included in the sponsor's NDA submission:

Species (duration)	Dose (mg/kg/day)	C_{max} (μg/ml)		AUC_{0-24h} (μg-hr/ml)		Margin Over Human[l]	
		Ro 64-0796	Ro 64-0802	Ro 64-0796	Ro 64-0802	Ro 64-0796	Ro 64-0802
Mouse (3 months)	100	8.19	12.6	9.43	30.4	36	5.2
	250	16.50	27.5	34.9	108	130	19
	600	21.80	41.3	90.1	249	350	43
	1000	39.50	50.6	250	421	960	73
Rat[a] (6 months)	50	5.80[j]		13.9[j]		13[k]	1.8[k]
	100	10.2[j]		34.3[j]		33[k]	4.4[k]
	200	20.2[j]		83.2[j]		80[k]	11[k]
	1000	57.5[j]		544[j]		520[k]	70[k]
Juvenile rat[b] (4 weeks)	50	0.887	1.48	2.69	8.48	10	1.5
	150	3.33	5.91	16.2	26.9	63	4.6
	500	6.88	15.4	36.7	103	140	18
Pregnant rat[c] (2 weeks)	500	11.9	20.0	104	214	400	37
	1500	29.9	80.4	422	1040	1600	180
Pregnant rabbit[d] (13 days)	50	5.75	7.24	9.62	17.0	37	2.9
	150	9.31	17.5	20.2	45.8	78	7.9
	500	27.9	73.2	109	306	420	53
Marmoset[e] (9 months)	2 X 25[g]	1.55	6.47	3.69	25.1	14	4.3
	2 X 100[g]	3.63	27.2	11.3	116	44	20
	2 X 500[g]	10.2	93.2	52.4	580	200	100
Man (7 days)	1 X 75[h]	0.051	0.260	0.12±0.03	2.9±0.6		
	2 X 75[i]	0.059	0.370	0.26±0.08	5.8±1.1		
Child[f] (1 day)	2 X 2	0.064	0.284	0.32	5.5		

a Mean of 3 and 6 month data
b Weaned juvenile rats aged 3-7 weeks
c Final day of dosing on gestation day 21
d Final dosing day on gestation day 18
e Mean of all data (5 collection days)
f Children aged 5-8 years old
g Daily doses divided into 2 doses given 2 hours apart
h Dose in mg, proposed prophylaxis regimen
i Dose in mg, proposed treatment regimen for this NDA
j Sum of Ro 64-0796 and Ro 64-0802 at time of sampling because of ex vivo hydrolysis of Ro 64-0796
k Margin of exposure over that of humans, calculated as $AUC_{animals}/AUC_{humans}$
l Magin calculated assuming 3:1 ratio of Ro 64-0796 and Ro 64-0802

OVERALL SUMMARY AND EVALUATION

Introduction:

The toxicity profile of Ro 64-0796 and the active metabolite, Ro 64-0802, have been fully explored as per the ICH guidelines. The acute, subacute, and subchronic toxicities have been studied in rats, mice and marmosets up to 9 months. The studies to assess chronic toxicity and carcinogenicity are in progress in rats and mice. The toxic potential to many aspects of reproduction and the developing embryos, fetuses, and neonates has also been adequately investigated at dosages that gave systemic exposure >25 fold over human exposures. The genotoxicity of prodrug, the major and active metabolite, and several of the degradation products in the synthetic process had also been studied. Absorption, distribution, metabolism, and excretion (ADME) studies have also been conducted in various species. Except for a few toxicities noted in the general and reproductive toxicology studies, Ro 64-0796 appeared to be well tolerated in all species studied. The toxicities associated with GI system, kidneys, and bones and their clinical relevance will be discussed in the following section.

Clinical Relevance of Safety Issues:

The toxicities to GI system, kidneys, and bone observed in the nonclinical toxicology studies may have some clinical relevance. They will be discussed individually.

GI system [redacted] Ro 64-0796, was very irritating to the primate GI tract. 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. The drug had to be administered as 2 separate daily doses to reduce the drug-induced emesis and gastric irritation. Although it appeared to be less toxic to the rodent GI system, excessive postdosing salivation was observed in some reproductive toxicology studies. In clinical trials, vomiting, nausea, and abdominal pain occurred in a higher number of patients receiving 75 mg b.i.d. Ro 64-0796 as compared to those receiving placebo. Patients who received 150 mg b.i.d. in the phase I and II studies had worse GI-related adverse events. This toxicity will probably be dose-limiting in humans.

Kidneys Slight renal dysfunction manifested as slight plasma and urine electrolyte imbalance (less than 1-fold as compared to the controls) and slight changes of other clinical chemistry parameters (e.g., plasma urea nitrogen and creatinine levels) were evident in mice, rats, and monkeys. These changes did not worsen following long term (up to 9 months) drug administration. Some changes may have improved, suggesting adaptation. Chronic progressive nephropathy, corticomedullary mineralization, tubular mineralization (seen only in the one-month rat study), tubular vacuolation, basophilic tubules, and focal nephropathy were observed in rodents. However, the incidence and severity of these histopathological changes did not worsen and remained 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. It has been suggested by an expert pathologist consulted by the sponsor that these renal changes in rats may be a result of

the high content of phosphate in the Ro 64-0796/002 [redacted] that would negatively influence the dietary calcium/phosphate ratio in a species known to be sensitive to this kind of change. Thus, the histopathological changes seen in rats, he reasoned, must be species-specific. However, other explanations 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 who do not convert prodrug to active metabolite as efficiently. However, there is a 5-10 fold of safety margin since humans hydrolyze prodrug 5-10 fold more efficiently as compared to rats. All of the renal changes in animals were reversible after a period of drug-free recovery period.

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. They may be attributed the pre-existing osteomalacia. However, slightly but statistically significant elevations of alkaline phosphatase levels were detected in high dose animals (≥ 1000 mg/kg/day) in a few rat toxicity studies. In addition, incomplete or no ossification of various bones and various other minor bone abnormalities and variants were observed in both rats and rabbit fetuses exposed to Ro 64-0796 *in utero*. These bone findings in the reproductive toxicity studies will be included in the Label. All of these bone-related findings suggest that Ro 64-0796 and/or its active metabolite may have some effect on the ossification process. The bone effect may have less clinical relevance in the treatment regimen but may be of clinical concern in a prophylaxis regimen.

Juvenile The drug exposure to prodrug in the neonate (4-21 days old) rats was ~ 6-10-fold higher than in adult and weaned (3-7 weeks old) rats. There were no differences in the toxicity profile between the adults and weaned/unweaned juvenile rats. However, the juvenile rats, especially the unweaned ones, tolerated lower doses of Ro 64-0796. It was suggested that infants and children under 5 (the age range not studied clinically) may have lower tolerance to the drug than adults and older children.

Conclusions:

Ro 64-0796 and its active metabolite, Ro 64-0802, are generally well tolerated and have a good safety profile at fairly high dosages and systemic exposures in all nonclinical animal species studied. The results from the nonclinical pharmacology/toxicology studies do not raise any clinical safety concern for the proposed treatment regimen (75 mg b.i.d. for 5 days) except perhaps during pregnancy and breast-feeding and for very young children. The bone abnormalities associated with rat and rabbit fetuses exposed to the drug *in utero* will be communicated in the drug Label. GI irritation is apparent and detected in clinical trials. This toxicity will probably be dose-limiting. The relationship between bone toxicity and drug exposure and duration of administration is unclear. However, the data do suggest a link of bone effect to Ro 64-0796 administration. Finally, the renal toxicity may be a concern for severely

effect to Ro 64-0796 administration. Finally, the renal toxicity may be a concern for severely hepatic impaired patients who will take this drug prophylactically. The safety margin for this toxicity is 5-10 fold for healthy patients.

/S/

Ita Yuen, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/WDempsey /S/ 10/27/99
HFD-530/JFarrell /S/ 10/21/99

cc:

HFD-530/NDA 21,087 (ori)
HFD-530/Division File
HFD-530/GCarmouze
HFD-345
HFD-530/TWu
HFD-530/NBattula
HFD-530/DBoring
HFD-530/Prajagopalan
HFD-530/THammerstrom