Table 2

Summary of rat (n=4) pharmacokinetics parameters for PMPA prodrugs

Species	Dose (mg-eq PMPA/kg	T_{max} (hr)	$T_{½}$ (hr)	Cmax (µg/ml)	$AUC_{0-\infty}$ (µg*hr/ml)	F (%)
bis-(Et COM) PMPA	10	0.94	11.4	0.06	0.514	4.31
bis-POCPMPA	10	0.75	6.48	0.228	1.4	13.7

4. Pharmacokinetics and oral bioavailabilities of PMPA prodrugs GS1480 and GS4331 in beagle dogs (96-DDM-1278-007)

The oral bioavailability of two prodrugs of PMPA (GS1278) were determined in male beagle dogs. The two prodrugs were bis-(pivaloyloxymethyl) PMPA or bis-(POM) PMPA, GS1480 and bis-POCPMPA, GS4331. The two prodrugs were dosed at 7.5 and 9.5 mg-equiv of PMPA per kg, for bis-(POM) PMPA and bis-POCPMPA, respectively. Plasma samples were obtained over the course of 24 hr and concentrations of each drug were determined by a validated HPLC method. The concentrations were compared to data for iv PMPA in the same species. Results: are summarized in Table 3. No intact prodrug or monoester were observed in dog plasma following oral administration of bis-(POM) PMPA. A monoester (3.1%) was observed following the oral administration of bis-POCPMPA.

Table 3

Summary of dog (n=5) pharmacokinetics parameters for PMPA prodrugs

Species	Dose (mg-eq PMEA/kg	T_{max} (hr)	$T_{½}$ (hr)	Cmax (µg/ml)	% Dose excreted in 48 hr	$AUC_{0-\infty}$ (µg*hr/ml)	F (%)
bis-(POM) PMPA	7.5	0.75	14.2	2.69	31.1	11.1	37.8
bis-POCPMPA	9	0.7	16.2	2.58	21.1	10.5	30.1

5. Single dose oral bioavailability of bis-POCPMPA in beagle dogs (D2000076)

In a single dose three way cross-over design study, tenofovir

plasma pharmacokinetics were evaluated in adult male beagle dogs (8) following: 1) single dose iv bolus administration of tenofovir at doses of 1 and 2 mg/kg; 2) single dose oral administration of 5 and 30 mg/kg tenofovir DF in the fed state; and 3) single oral dose administration of 5 and 30 mg/kg tenofovir DF in the fasted state. Plasma samples were obtained over the course of 120 hr postdose and concentrations of PMPA were determined by a validated HPLC method. Results: are summarized in Tables 4 and 5. Tenofovir DF was adequately absorbed in this species. There were no statistically significant effect of food the pk parameters.

Table 4
Mean pharmacokinetic parameters of PMPA following iv administration of PMPA (1 or 10 mg/kg) to male dogs

Parameters	1 mg/kg)	10 mg/kg
AUC (µg*hr/ml)	4.48	41.2
Cl (ml/hr/kg)	226	244
Vss (ml/kg)	3090	2510
T½ (hr)	45.3	38.6
Bioavailability	100	100
Cmax (µg/ml)	2.99	31

Table 5
Mean pharmacokinetic parameters of PMPA following a single oral administration of bis POC PMPA (5 or 30 mg/kg) to male dogs

Parameters	5 mg/kg)		30 mg/kg	
	Fasted	Fed	Fasted	Fed
AUC (µg*hr/ml)	2.68	2.69	21.4	23.6
Cl/F (ml/hr/kg)	762	752	608	563
Tmax (hr)	1.38	1.13	1.13	0.65
T½ (hr)	78.5	81.3	45.8	49
F (%)	30.2	31.8	41.2	46.4
Cmax (µg/ml)	0.4	0.49	3.49	4.69

6. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum, Cidofovir Lot # 1966-C-9P), Gilead Sciences, Inc., Foster City, CA, June 9, 1995 (PO504-00039/95-DDM-XXXX-001)

The protein binding of cidofovir, cyclic HPMPC, 9-(2-phosphonylmethoxyethyl) adenine [PMEA] and 9-(2-phosphonylmethoxyproyl) adenine [PMPA] was determined in human plasma and serum using a centrifugation and ultrafiltration method. Five concentrations of each compound were prepared in phosphate buffered saline (PBS), human plasma and serum over the range of 0.6 to 25.6 µg/ml for cidofovir and cyclic HPMPC, 0.1 to 25.1 µg/ml for PMEA and 0.01 to 25.01 for PMPA. Results: are shown in Table 6. All the four compounds showed a very low protein binding (< 6%) in either human plasma or serum.

Table 6
Percent Unbound (SD) Cidofovir, Cyclic HPMPC, PMEA and PMPA in

Compound	Mean Percent Unbound (SD)		
	Human Plasma	Human Serum	PBS
Cidofovir	94.8 (3.8)	99.9 (3.3)	100.6 (1)
Cyclic HPMPC	96.7 (3.9)	95.7 (5.2)	99.3 (4.2)
PMEA	98.2 (6.3)	100.8 (7.2)	100 (1.3)
PMPA	99.3 (3.3)	92.8 (3.6)	99.8 (2.3)

7. Determination of bis-POCPMPA and metabolite concentrations in bile and gastrointestinal tract, following oral administration of bis-POCPMPA to rats, June 15, 2000, (97-DDM-4331-003)

A bile duct catheterized male Sprague-Dawley rat was administered 10 mg-equiv. of tenofovir/kg (containing 25 µCi ^{14}C-tenofovir disoproxil fumarate (DF)/kg in 50 mM citric acid buffer, pH = 2) via oral gavage. Bile fluid samples were collected at predose, 0.5, 2.2, 3, 4, 5, 21.5, 22.5 and 24 hr postdose. Concentrations of total radioactivity in bile were determined by direct scintillation counting. Results: are summarized in Table 7. The total dose excreted in the bile was 0.12%. Tenofovir was the major radioactive species detected in the bile samples. Tenofovir soproxil (the monoester) was detected in the 0.5-2.2 hr time point sample (37%).

Table 7
The concentration, percentage of dose recovered and metabolite in bile samples following oral administration of ^{14}C-Tenofovir disoproxil fumarate to rats (10 mg-equiv. of tenofovir/kg)

Time Period (Hr)	Total radioactivity (µg-equiv. of tenofovir/ml)	% of dose	Metabolites in bile (HPLC)

0	nd	0	nd
0-0.5	nd	0	nd
0.5-2.2	0.66	0.014	tenofovir (63%) tenofovir soproxil (37%)
2.2-3.0	0.53	0.009	tenofovir
3.0-4.0	0.51	0.009	tenofovir
4.0-5.0	0.31	0.005	tenofovir
5.0-21.5	0.065	0.002	nd
21.5-22.5	0.065	0.002	nd
22.5-24.0	0.267	0.076	nd

nd = not detected

In a second study, a fasted male Sprague-Dawley rat was administered 10 mg-equiv. of tenofovir/kg (containing 25 µCi ^{14}C-tenofovir DF/kg in 50 mM citric acid buffer, pH = 2) via oral gavage. Gastrointestinal contents and tissues were harvested 1 hr postdose. Stomach, intestinal tissues and contents were processed and concentrations of total radioactivity were determined by direct scintillation counting. Results: are summarized in Table 8. Approximately 65% of the total dose was recovered in gastrointestinal contents 1 hr post dose. Intact tenofovir DF (prodrug) was the major radioactive species (>85%) detected in the stomach and its contents.

Table 8

The percentage dose remaining and metabolite profile at 1 hr following oral administration of ^{14}C-Tenofovir disoproxil to rats (10 mg-equiv. of tenofovir/kg)

GI contents	Metabolic profile (% of total peak area)	Amount of radioactivity remaining at (1 hr)

	Tenofovir disoproxil	Tenofovir soproxil	Tenofovir	µg-equiv. tenofovir	% dose
Stomach	85.3	8.1	6.6	138	4.5
Duodenum	nd	nd	100	27	0.9
Jejunum	nd	nd	100	124	4
Ileum	nd	28.2	71.8	1707	55.1
Cecum	nd	nd	nd	nd	0
Colon	nd	nd	nd	nd	0
Total				1996	64.5

nd = not detected

Comments: In this study, the biliary excretion of tenofovir DF in rats over 24 hr post dose following the oral administration was negligible. At 1 hr post dose, the majority of the dose remained in the intestinal tract, particularly the ileum. Intact tenofovir DF was the major radioactive species detected in the stomach and its contents; whereas, tenofovir was the major metabolite detected in the small intestinal tissues and contents.

8. Oral bioavailability of PMPA from bis-POCPMPA in Rhesus monkey (P4331-00017.1)

Two male and female monkeys received single doses both intravenous PMPA (10 mg/kg) and oral (gavage) bis-POCPMPA (10 mg-equiv. of PMPA/kg). Plasma samples were obtained over the course of 24 hr and concentrations of PMPA were determined by a validated HPLC method. Urine samples were collected over 0-24, 24-48 and 48-72 hr post dose and were analyzed for PMPA concentrations. Results: are summarized in Tables 9 and 10. The concentrations of PMPA declined in bi-exponential manner. Clearance (0.71 L/hr/kg was higher than GFR in the animal. The urinary recovery results are shown in Table 10.

Table 9

Mean pharmacokinetic parameters of PMPA following iv administration of PMPA (10 mg/kg) and oral administration of bis-POC PMPA (10 mg/kg) to Rhesus monkey

Parameters	PMPA, iv, (10 mg/kg)	bis-POC PMPA, oral, (10 mg/kg)
AUC (µg*hr/ml)	14.7	0.71
Cl (L/hr/kg)	0.71	-
Tmax (hr)	-	1.5
T½ (hr)	11.1	-

Bioavailability	100	4.9
Cmax (µg/ml)	-	0.13

Table 10
Urinary recovery of PMPA in 72 hr following iv administration of PMPA (10 mg/kg) and oral administration of bis-POC PMPA (10 mg/kg) to Rhesus monkey

Compound	% dose			total
	0-24 hr	24-48 hr	48-78 hr	
iv PMPA	32.9	25.6	5.41	63.9
oral bis-POC PMPA	3.44	3.84	1.3	8.58

9. Epithelial transport and metabolism of bis-POCPMPA in Caco-2 cell monolayers, June 8, 2000, (98-VIT-4331-001)

Caco-2 cells (colon, adenocarcinoma; human) were obtained from the American Type Culture Collection at passage 17 to study epithelial transport and metabolism of tenofovir disoproxil fumarate in vitro. Cells were seeded at a density of 63,000 cells/cm^2 onto Transwell^R polycarbonate membrane filters. Tenofovir disoproxil (100 µM) was incubated with Caco-2 cell monolayers for a period of 60 min. Results: tenofovir disoproxil, tenofovir soproxil and tenofovir were detected inside the monolayer following the incubation. Tenofovir soproxil was the major intracellular metabolite observed.

10. A 13-week oral gavage toxicity study of bis-POCPMPA in the albino mice, Lot # 2454-A-2P, Clinical Trials Bioresearch, Sennebille, Quebec, Canada, August 18, 2000, (Project No. 89283/Gilead Study No. M990203, Toxicokinetic data)

Groups of male and female albino mice [weight: 21.9-32.3 g; strain: Crl:CD-1(ICR)BR] were orally gavaged with bis-POCPMPA suspension (suspension vehicle lot # TX4331-99-01; dose volume = 10 ml/kg/day) at dose levels shown in Table 11 daily for a period of 13 consecutive weeks.

Table 11
Study design of the 13-week oral gavage toxicity study of tenofovir in the albino mouse

Dose Level (mg/kg/day)	Group	Number of animals			
		Main Study		Toxicokinetic Group	
		Males	Females	Males	Females
0	Vehicle Control	15	15	-	-
100	Low	15	15	32	-
300	Mid	15	15	32	-
1000/600*	High	15	15	32	32
600**	High	-	-	16	16

* = dose level reduced 1000 mg/kg/day to 600 mg/kg/day on day 9 of the study due to high mortality seen at the 1000 mg/kg/day dose level

** = for toxicokinetic purposes only to obtain day 1 information at 600 mg/kg/day dose level

For toxicokinetic analyses, blood samples (approximately 1 ml) were taken at 0, 5, 15, 30 minutes, 1, 2, 4, 8 hr post-dose on days 1 and 91. Plasma samples were analyzed for tenofovir by a validated HPLC/fluorescence method. Results: Pharmacokinetic parameters estimated by non-compartmental analysis are shown in Table 12. The limited data obtained precluded any statistical analysis comparing day 1 results with those from day 91, and it was not possible to draw any conclusions based on the sex of the animals. Overall, tenofovir Cmax AUC appeared to increase in a dose proportional manner. Comparative human and mouse exposure data for tenofovir are summarized in Table 13. Based on these results, data from mice dosed at 600 mg/kg/day would be expected to provide a safety margin of approximately 22.3-fold for humans administered 300 mg/day of tenofovir.

Table 12

Mean plasma pharmacokinetic parameters of tenofovir following repeated oral administration of tenofovir to mice for 13 weeks

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
100	day 1, ♂	55	3.6	0.25	42.5
	day 91, ♂	15.5	9.39	0.08	10.2
300	day 1, ♂	49.1	6.26	0.5	16.3
	day 91, ♂	36	19.1	0.08	10.8

600	day 1, ♂	31.9	26.4	0.08	5.05
	day 1, ♀	51	22	0.5	3.24
	day 91, ♂	72.4	30.5	0.25	72.4
	day 91, ♀	58.7	41.4	0.08	58.7
1000	day 1, ♂	39.9	18.4	0.05	1.8
	day 1, ♀	50.1	56.2	0.08	3.06

Table 13

Comparative human and mouse exposure data for tenofovir

Species	Dose level	Sampling time point	AUCss (µg*hr/ml)	Reference
Mouse	100 mg/kg/day	day 91	15.5	M990203
	300 mg/kg/day		36	
	600 mg/kg/day		65.6*	
Human	150 mg/day	day 15	1.64	DDM-GS-97-901
	300 mg/day		2.94	
	600 mg/day		6.07	

* = combined values, males and females

11. Determination of PMPA in plasma samples from a 14-day oral gavage toxicity study of bis-POCPMPA Fumarate in the albino rat (98-TOX-4331-004/E748-07)

Groups of male and female Sprague-Dawley CD rats were administered GS-4331-05 via oral gavage at dose levels of 0 (vehicle control), 500 (low), 750 (mid), 1000 (high) or 1250 mg/kg/day (very high) for a period of 2 weeks. Blood samples were collected at one hr after dosing on days 1 and 14 of the study. Plasma samples were analyzed for PMPA concentrations using a validated HPLC assay. Results: mean plasma concentrations from the 2-week toxicity rat study are shown in Table 14.

Table 14

Mean plasma concentrations of PMPA from the 2-week oral gavage study of bis-POCPMPA in rats

Dosage levels (mg/kg/day)	day 1 (µg/ml)	day 14 (µg/ml)
500 (low)	1.78	1.48
750 (mid)	1.71	2.19
1000 (high)	2.07	3.29
1250 (very high)	2.76	no sample

12. Pharmacokinetics of bis-POCPMPA in a 28-day toxicity study in albino rats (96-TOX-4331-003-PK) and analysis of PMPA in rat plasma for a 28-day oral gavage toxicity study of bis-POCPMPA (P4331-00004)

Groups of male and female albino rats were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 20 (low; 8 ♂), 100 (mid; 8 ♂) or 500 mg/kg/day (high; 8 animals/sex/group) once daily for 4 consecutive weeks. For toxicokinetic analyses (2 animals/sex/time point), blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose on day 1 and week 4. Tenofovir plasma concentrations were determined by a validated reverse-phase HPLC method with fluorescence detection. Results: are shown in Table 15. The pharmacokinetics of tenofovir in rat appeared to be dose-dependent based on Cmax. Following oral administration of tenofovir on day 1, Cmax deviated from dose proportionality over the range of 20 to 500 mg/kg/day, indicating decreased absorption at the higher dose. Conclusion: repeated administration of tenofovir over a 28 days period resulted in only minor changes in pharmacokinetics.

Table 15

Mean plasma pharmacokinetic parameters of tenofovir following repeated oral administration of tenofovir to rats for 4 weeks

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
20	day 1, ♂	nd	0.2	1	-
	day 28, ♂	nd	0.22	1	-
100	day 1, ♂	nd	0.85	0.5	-
	day 28, ♂	nd	0.83	0.5	-
500	day 1, ♂	8.93	1.91	0.25	31.6
	day 1, ♀	6.56	1.13	0.25	21.5

	day 28, ♂	13.7	2	0.5	10.1
	day 28, ♀	17	1.57	0.5	10.4

13. A 28-day study to evaluate the effects of bis-POCPMPA on bone following daily administration by gavage in the Sprague-Dawley rat (PK portion), Lot # 2454-A-10P, ClinTrials BioResearch LTD., Senneville, Quebec, January 12, 2001, (R2000036/T4331-00022)

Groups of male Sprague-Dawley rats [weight: 172-220 g; strain: Crl:CD(SD)BR; 10-31 animals/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 40 (low) or 400 mg/kg/day (high) once daily for 28 days to evaluate the effects of tenofovir on biochemical markers of bone turnover. Blood samples were collected from 3 animals/treated group on study days 1, 13 and 28 at 0, 0.25, 1, 1.5, 2, 3, 4, 6, 12, 18 and 24 hr after dosing, and were analyzed by a validated HPLC method for tenofovir concentrations. Results: pk values are shown in Table 16. Repeated administration of tenofovir over a 28 days period resulted in only minor changes in pharmacokinetics relative to day 1.

Table 16

Mean pharmacokinetic parameters of tenofovir in plasma following repeated oral administration of tenofovir to male rats for 28 days

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
	-	$AUC_{0-\infty}$ (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
40	day 1	2.39	1.06	0.25	6.88
	day 13	2.53	0.77	0.5	6.82
	day 28	3.13	1	0.5	8.34
400	day 1	9.67	3.17	0.5	19.7
	day 13	11.9	2.55	0.5	13.5
	day 28	19.6	4.01	1	21.8

14. Pharmacokinetics of tenofovir in a 13 and 42-week oral gavage toxicity study (with a 13-week recovery period) of tenofovir in the albino rat, May 8, 2000, (97-TOX-4331-002-PK)

Groups of male and female Sprague-Dawley rats [weight: 128-220 g;

strain: Crl:CD(SD)BR; 20-27 animals/sex/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 30 (low), 100 (mid), 300 (high) or 1000 mg/kg/day (very high) once daily for 13 or 42 consecutive weeks followed by a 13-week (5 animals/sex/group) drug free recovery period (week 55). For toxicokinetic analyses (2 animals/sex/time point), blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose on days 1 and at weeks 13, 26 and 42. Tenofovir plasma concentrations were determined by a validated reverse-phase HPLC method with fluorescence detection. Results: are shown in Table 17. Compared to day 1, mean plasma AUCs at the 30, 100, and 1000 mg/kg/day doses were increased by 32%, 25% and 49%, respectively following 42 weeks of daily dosing. At the 300 mg/kg/day dose, no change in AUC was observed following 42 weeks of daily dosing. Conclusion: No definitive dose-dependent changes were observed in the pharmacokinetics of tenofovir following 42 weeks of daily oral dosing of tenofovir to rats.

Table 17

Mean pharmacokinetic parameters of tenofovir in plasma following repeated oral administration of tenofovir to rats

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
30	day 1	2.87	0.46	0.38	6.21
	week 13	2.65	0.56	0.75	4.93
	week 26	3.93	0.66	0.5	8.3
	week 42	3.78	0.74	0.5	7.24
100	day 1	6.64	1.17	0.75	10.7
	week 13	7	1.4	0.75	7.7
	week 26	6.89	1.36	0.5	8.9
	week 42	8.34	1.86	1	9.34
300	day 1	17.7	1.51	0.75	17.3
	week 13	18.4	2.86	2.5	7.21
	week 26	13.8	2.32	1	11
	week 42	17.6	2.18	1	9.1
1000	day 1	43.5	2.71	0.75	29.6
	week 13	34.1	4.52	0.5	25.6
	week 26	42.8	5.82	1	12.1

	week 42	64.85	6.57	1.5	9.66

15. Four week oral gavage toxicity study of bis-POCPMPA in the beagle dog (PK portion) (98-TOX-4331-003-PK)

Groups of male beagle dogs (4/group) were orally gavaged with bis-POCPMPA at dose levels of 0 (bid, vehicle control), 15 (bid, low), 30 (qd, mid), 60 (q2d, high) or 210 mg/kg/dose (q7d, very high) over a 4-week period to investigate the potential toxicity of the test article during daily, twice daily, every other day or weekly administration to dogs. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 12 and 24 hr postdose on days 1, 2, 8, 15, 16, 22 and 28. The plasma samples were analyzed by a validated HPLC method. Results: are shown in Table 18. Comparison of dose normalized Cmax suggested a decrease in Cmax with increasing dose that appeared to correspond with a delay in Tmax.

Table 18

Mean plasma pharmacokinetic parameters of tenofovir following repeated oral administration of tenofovir DF to dogs for 4 weeks

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T1/2 (hr)
15 bid	day 1,	5.2	2.64	0.5	3.37
	day 28,	nd	2.62	0.875	-
30 qd	day 1,	12.1	4.48	0.5	13.4
	day 28,	29.4	7.54	0.75	29.4
60 q2d	day 1,	24.1	8.04	0.75	17.1
	day 28,	67	13	1.38	41.6
210 q7d	day 1	127	26.9	0.75	42.5
	day 28	219	32.9	1	40.1

16. Pharmacokinetics of bis-POCPMPA in a 13- and 42-week repeat dose toxicity study in beagle dogs (97-TOX-4331-001-PK)

Groups of male and female beagle dogs (weights: 8.4-12.4 kg) were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 3 (low), 10 (mid) or 30 mg/kg/day (high) once daily for 13 (4 dogs/sex/group) or 42 (4 dogs/sex/group) consecutive weeks followed by a 13-week (2 dogs/sex/group) drug free recovery

period (week 55). Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose on days 1 and weeks 13, 26 and 42 to assess systemic drug exposure. Results: are shown in Table 19. Tenofovir Cmax appeared to increased in a dose linear manner following day 1 of administration. Following repeat dosing, AUC values increased from 11.5 µg*hr/ml on day 1 to steady state levels of 29 to 29.8 µg*hr/ml at weeks 13 and 42 at the high dose cohort. These data suggested nonlinear pharmacokinetics upon repeated dosing in the dog.

Table 19

Mean pharmacokinetic parameters of tenofovir in plasma following repeated oral administration of tenofovir to male and female dogs for 42 weeks

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
3	day 1	nd	0.25	0.5	-
	week 13	nd	0.22	0.75	-
	week 26	nd	0.26	1	-
	week 42	2.12	0.29	1	34.4
10	day 1	nd	0.66	0.5	-
	week 13	5.73	1.37	0.5	27.3
	week 26	5.92	1.01	0.75	40.6
	week 42	6.63	1.4	0.5	37.9
30	day 1	11.5	2.46	0.5	18.8
	week 13	29.3	7.36	0.75	31.4
	week 26	29.8	8.15	0.75	22.8
	week 42	29	7.52	1	28.2

17. Pharmacokinetics of tenofovir in an oral (stomach tube) developmental toxicity study of bis-POCPMPA in Rabbits (98-TOX-4331-005-PK)

Groups of female New Zealand White rabbits [Hra:(NZW)SPF; weights: 2.5-3.5 kg; 25-35 animals/group] were orally

administered (stomach tube) bis-POCPMPA at dose levels of 0 (vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) once daily on days 6-18 of presumed gestation. Blood samples were collected on gestation day 18 at 0, 0.5, 1, 2, 4, 8, 12 and 24 hr postdosage. The plasma samples were analyzed by a validated analytical method. Results: are shown in Table 20.

Table 20
Mean pharmacokinetic parameters of PMPA following oral administration of bis-POC PMPA to rabbits (n=4) on day 18

Parameters	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
AUC (µg*hr/ml)	19.15	65.3	212.48
Cl/F (ml/hr/kg)	740	730	720
Tmax (hr)	0.75	0.5	0.75
T½ (hr)	8.61	10.34	9.67
Cmax (µg/ml)	4.92	20.65	47.62

18. Comparison of plasma pharmacokinetics in rats of tenofovir following oral administration GS-7340-02 or tenofovir DF as either a suspension in carboxymethylcellulose or a solution in citric acid (P7340-00001/R2000065)

Single dose plasma pharmacokinetics of tenofovir was evaluated in male Sprague Dawley rats following a 400 mg/kg oral dose of GS-7340-02 (a prodrug of tenofovir) in citric acid, GS-7340-02 in carboxymethylcellulose, tenofovir DF in citric acid and tenofovir DF in carboxymethylcellulose. Plasma samples were analyzed for tenofovir using a validated LC/MS/MS assay. Results: are shown in Table 21. Plasma PK profiles reveled no significant differences between delivery vehicles. Also, secondarily, significant differences were observed for plasma profiles of GS-7340-02 and tenofovir DF.

Table 21
Plasma tenofovir pharmacokinetic parameters following a single 400 mg/kg oral dose of GS-7340-02 or tenofovir DF in different formulations.

Formulations	Pharmacokinetic parameters			
	$AUC_{0-\infty}$ (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
GS-7340-02 in citric acid	33.0	8.41	0.25	11.4
GS-7340-02 in carboxy methylcellulose	36.2	14.2	0.5	11.3
Tenofovir in citric acid	11.4	2.69	0.5	8.31
Tenofovir in carboxy methylcellulose	15.7	8.10	0.25	7.21

19. Bis-POC PMPA: Spectrum screen, Gilead Sciences, Inc., Boulder, CO, October 10, 2000, (T43331-00019/V2000020)

This study examined the ability to inhibit or stimulate binding of ligands to their respective proteins. This assay preformed a primary screen test with bis-POC PMPA in a series of 111 protein targets (neuroreceptors, ion channels, transporters and nuclear receptors) in the presence of 10 µM concentration of the test compound. Results: the test compound showed no significant inhibition or stimulation of ligand binding to its protein target. Conclusions: these results suggest that bis-POC PMPA did not significantly interact with the protein targets (neuroreceptors, ion channels, transporters and nuclear receptors) tested in the assay.

20 Tissue distribution of ^{14}C-tenofovir DF in beagle dogs following oral administration (P4331-00026)

Three groups of male and female dogs (2 animals/sex/group) were orally gavaged with a single dose of ^{14}C-tenofovir DF (10 mg/kg). Tissue distribution and excretion of the test article was examined at 1, 6 and 24 hr postdose. Results: 1-hr post dose, radioactivity was detected in all tissues and organs examined, with the exception of the brain. The highest concentrations of radioactivity, excluding the GI tract contents, were found in the bile, kidneys and jejunum. Six hr postdose, concentrations of radioactivity were notably decreased in all tissues and organs examined, with the exception of the large intestinal contents. Within 24 hr, the concentration of the radioactivity was greatly reduced in the majority of the organs and tissues examined. Of the samples examined, the kidneys, liver, large intestinal contents and small intestinal contents had the highest concentrations of radioactivity. Analysis of the excreta from the 24 hr group indicated that approximately greater than 60% of the total radioactivity was eliminated during the study period. The majority of the radioactivity was eliminated via the feces (>34%); elimination via the kidney was 24%.

Tenofovir

21. In vitro metabolism of ^{14}C-PMPA in human and animal tissues (96-DDM-1278-003)

In an in vitro experiment, ^{14}C-PMPA (5 mg; 10 µCi) was incubated with plasma and microsomes from rat, dog and human tissues (liver and intestine) at 37°C for 60 min to evaluate the stability of the compound. The assay (rat only) was conducted in the presence and absence of a metabolic activation system using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. Concentrations of PMPA were determined using a chiral HPLC assay. Radioactivity associated with the protein pellet was determined by sample oxidation and liquid scintillation. Results: no metabolites were detected in either rat microsomal fraction, with or without the S9 fraction. PMPA was recovered unchanged and less than 0.1% of the radioactivity was associated with the protein pellet. There was no evidence of chiral inversion. The stability of PMPA was further confirmed in the human and dog tissues tested. Conclusions: the results of these studies indicated that PMPA was metabolically stable.

22. Determination of distribution of ^{14}C-PMPA in male Sprague-Dawley rats following single administration using whole body autoradiography (95-DDM-1278-002)

Two male Sprague-Dawley rats were administered ^{14}C-PMPA (28 µCi) as a single 10 mg/kg one each either oral or iv dose to investigate the tissue distribution of total radioactivity by using qualitative whole body autoradiography. At 24 hr post-dose, the animals were sacrificed by carbon dioxide inhalation. Rats were processed, sectioned and autoradiographs were produced after a 14-, -28 and 56-day exposure. Results: distribution of ^{14}C-PMPA was limited following both routes of administration at the 24-hr time point. The iv route showed the most distribution, with the kidney, urine and large intestinal contents containing the highest levels of radioactivity. The highest radioactivity concentrations were found in the contents of the mid to lower GI tract. Low levels of radioactivity were also found in the kidney and urine. Evaluation of 56-day exposures revealed very low levels of radioactivity in the spleen, pancreas, brown fat, lymph nodes, bones and bone marrow of the iv dosed animal, while the orally dosed animal displayed very low levels of radioactivity in the stomach contents. Conclusions: the iv dosed animal had markedly higher levels of radioactivity in the liver, kidney and urine than the orally dosed rat.

23. Effect of dose on the recovery of ^{14}C-PMPA following iv administration to Sprague-Dawley rats (96-DDM-1278-002)

Groups of male Sprague-Dawley rats (4/group) received a single iv

dose of ^{14}C-PMPA (400 µCi/kg) at dose levels of 10 (low) or 50 mg/kg (high) to study the effect of dose on excretion and metabolism of PMPA. Urine, cage wash and feces samples were collected at predetermined time-points over a 7 day period. Total radioactivity was determined by sample oxidation and liquid scintillation counting. Urine and feces samples were further analyzed for the presence of metabolites using HPLC with radioactive flow detector. Results: the mean cumulative recovery of radioactivity in the urine/cage wash was 85.2% by 24 hr and 92.7% by 7 days post-dose (low). The mean terminal elimination half-life from urine data was 15.82 hr. PMPA was the only species present in the urine and feces; no metabolites were detected. The mean recovery of the administered dose in the feces was 3.18% by 24 hr and 4.48% by 7 days post-dose. At the high dose, the mean cumulative recovery of the administered dose in urine/cage wash was 77.5% by 24 hr and 84% by 7 days post-dose. The mean terminal half-life from urine data was 20.38 hr. The corresponding mean recovery of the administered dose in the feces was 7.39% by 24 hr and 8.46% by 7 days post-dose.

Comments: These results suggested that, at 10 and 50 mg/kg dose levels, PMPA was primarily excreted by renal clearance of the unchanged drug. At the high dose, renal clearance appeared to be partially saturated and fecal recovery increased (~2-fold). The non-linear pharmacokinetics of PMPA in the rat appeared to be a consequence of saturation of active tubular secretion.

24. A pilot study of biliary excretion of ^{14}C-PMPA in beagle dogs (96-DDM-1278-002)

One male dog was surgically cannulated to permit the continuous collection of bile via the common bile duct. The animal received a single iv administration of ^{14}C-PMPA at a dose level of 10 mg/kg (50 µCi/kg). Total bile, urine and feces outputs were collected at 0-4, 4-8, 8-12, 12-24 and 24-48 hr post-dose. Results: the treatment appeared to be tolerated by the dog as indicated by the absence of abnormal animal observations. The primary route of elimination was via the kidneys, as 70.03% of the total unchanged dose was recovered in the urine during the first 48 following dosing. Biliary recovery was at its highest during the 0-4 hr post-dose period (0.07%), and generally decreased steadily for the duration of the study period. Total biliary recovery was 0.26%; total fecal recovery was 0.42%.

25. Placental transfer and pharmacokinetics of PMPA in infant Cynomolgus monkeys (96-DDM-1278-005)

Groups of infant Cynomolgus monkeys (newborn, 1, 3 and 12 month old; .2/group) were administered sc PMPA at a single dose level of 30 mg/kg. Plasma samples were obtained over the course of 24 hr

and concentrations of PMPA were determined by a validated HPLC method. Additionally, placental transfer of PMPA following sc administration to a pregnant monkey was also determined. One monkey received daily sc injections of PMPA at a dose level of 30 mg/kg/day beginning gestational day 111. Maternal and fetal blood samples were drawn at day 115, 127, 134, 140 and 151. Results: are shown in Tables 22 and 23.

Table 22

Mean pharmacokinetic parameters of PMPA in infant Cynomolgus monkeys following sc administration at 30 mg/kg.

Parameter	Newborn	1 Month	3 Month	12 Month
Tmax (hr)	0.5	0.5	0.5	0.5
Cmax (µg/ml)	51.8	30.7	34.6	18.8
T½ (hr)	3.34	4.39	3.62	3.16
$AUC_{0-\infty}$ (µg*hr/ml)	164	58.4	77	30.1
MRT (hr)	3.17	2.01	1.93	1.41
CL/F (l/hr/kg)	0.18	0.544	0.406	1.02
Vdss/F (l/kg)	0.58	1.06	0.782	1.43

Table 23

Fetal and maternal serum concentrations of PMPA obtained at 30 min after sc administration at 30 mg/kg

Gestational day	Serum concentration (µg/ml)		Fetal/maternal ratio
	Fetal	Maternal	
115	7.9	45.6	0.17
127	9.1	61.2	0.15
134	10.1	69.4	0.15
140	15	53.7	0.28
151	5.9	56.3	0.11
Mean	9.6	57.2	0.17

Comments: The pharmacokinetics of sc PMPA in infant monkeys were age-dependent. At an equivalent dose, PMPA exposure decreased with increasing age of the monkeys. Similarly, an increase in clearance from birth through 1 year was observed. The clearance of PMPA in adult monkeys greatly exceeded the GFR in this species due to active tubular secretion. It is likely that newborn monkeys lack the anion transport system responsible for tubular secretion of PMPA. Placental transfer of PMPA appeared to be significant with a mean ratio of fetal/maternal serum concentrations of 0.17 at approximately 30 min post-dose.

26. Single dose iv pharmacokinetics of tenofovir in rats (R20.00075)

Male Sprague Dawley rats were administered a single iv bolus dose of either 10 mg/kg or 50 mg/kg tenofovir and plasma concentrations were evaluated using a validated HPLC method. Results: are summarized in Table 24. Tenofovir plasma concentrations were declined in a biphasic manner with an apparent terminal half-life of 4.02 to 5.41.

Table 24
Mean pharmacokinetic parameters of PMPA following iv administration of PMPA (10 or 50 mg/kg) to male rats

Parameters	10 mg/kg	50 mg/kg
AUC (µg*hr/ml)	5.86	53.7
Cl (ml/hr/kg)	1706	931
Vss (ml/kg)	2810	1122
T½ (hr)	4.02	5.41
Bioavailability	100	100
Cmax (µg/ml)	22	162

27. Intracellular kinetics of ^{14}C-PMPA in rhesus monkeys, March 20, 2001, (P2001025)

The systemic and intracellular pharmacokinetics of PMPA were determined in rhesus monkeys following single dose sc administration of PMPA at 15, 30 or 60 mg/kg. The plasma and intracellular PMPA mono- and diphosphate anabolites were determined in peripheral blood mononuclear cells (PBMC) and lymph node mononuclear cells (LMNC) and red blood cells following sc dosing of PMPA in rhesus monkeys. Concentrations of PMPA in plasma were well described by biphasic clearance with a terminal half life of approximately 8-10 hr. Accumulation of active anabolite, PMPA diphosphate in both PBMC and LMNC, with no apparent saturation of phosphorylation up to 60 mg/kg of administered dose was observed. The intracellular concentrations of PMPA diphosphate and it s immediate precursor, PMPA monophosphate, in PBMC and LMNC were similar and persisted in the cells in excess of 48 hr. The substantial concentrations of tenofovir anabolites for prolonged periods in lymphoid cells may provide an explanation for the efficacy of the test compound in HIV infected patients.

28. In vivo rat cytochrome P450 induction study following dosage with tenofovir DF (R2001024)

Groups of male Sprague-Dawley rats were orally gavaged with

tenofovir DF at dose levels of 0 (vehicle controls), 40 (low) or 400 mg/kg/day (high) for 28 days to induce the expression of selected rat liver cytochrome P450 enzymes. Livers were collected and microsomes were prepared separately from each liver. Phenacetin O-deethylase and testosterone 6 beta-hydroxylase activities were measured. These activities are mediated by CYP1A2 and CYP3A4 in humans. Results: activities of phenacetin O-deethylase and androstenedione formation were increased in the high dose group. The 6 beta-hydroxylation of testosterone in treated animals was not significantly different from controls.

SAFETY PHARMACOLOGY:

1. A pharmacological assessment of the effect of bis-POCPMPA on the renal system of the rat (P4331-00018)

Groups of male Sprague Dawley rats (strain: Crl:CD(SD) BR; 10 rats/group) were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0 (vehicle control), 50 (low) or 500 mg/kg (high) to evaluate the pharmacological effects. Results: there were no deaths; there were no treatment related clinical signs. There were no remarkable difference between creatinine clearance values for the control and treated groups. Serum concentrations of potassium and chloride were elevated (high). Urine: the volume of urine was significantly reduced, the quantities of excreted calcium, sodium, potassium, chloride and bicarbonate were decreased (high). Kidney weights both absolute and relative to body weight were reduced (high); there were no drug related gross pathology findings.

Comments: In this study, a dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person).

2. A pharmacological assessment of the effect of bis-POCPMPA on gastrointestinal motility in the rat (P4331-00019)

Groups of male rats (strain: CD (Crl:CD(SD)BR; 10 rats/group) were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0 (vehicle control), 50 (low) or 500 mg/kg (high) to observe the effects on the passage of activated charcoal along the GI tract; animals were sacrificed at 2, 4 and 6 hr after the treatment. Results: the increased weight of the stomach, and content and observation of charcoal in the stomachs (high) at all time points indicated that drug reduced the rate of gastric emptying. There were no changes at the low dose.

Comments: In this study, a dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an

equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person).

3. A pharmacological assessment of the effect of bis-POCPMPA on the central nervous system of the rat (P4331-00020)

Groups of male rats (strain: CD (Crl:CD(SD)BR; 10 rats/group) were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0 (vehicle control), 50 (low) or 500 mg/kg (high) to observe for apparent neuropharmacological signs following dosing. Results: group mean total activity counts (high) were statistically ($p<0.05$) reduced as compared to the controls.

Comments: In this study, a dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person).

4. A pharmacological assessment of the effect of bis-POCPMPA on the cardiovascular system of the beagle dog (P4331-00020)

A group of male beagle dogs under isoflurane/oxygen anesthesia (3 dog) were administered a single oral gavage dose of bis-POCPMPA (30 mg/kg) to determine effects on the cardiovascular/hemodynamic system over a period of 24 hrs. Results: there were no drug related effects on clinical signs, heart rate, systemic blood pressure or electrocardiograms.

Comments: In this study, a dose level of 30 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 16.2 mg/kg (973 mg/day for a 60 kg person).

MOLECULAR PHARMACOLOGY:

The following summaries were provided from the published scientific literature.

Mechanism of action of PMPA - DNA polymerase inhibition

The putative active metabolite of PMPA is PMPApp. PMPApp competitively inhibited both RNA- and DNA- directed reverse transcriptase activities. PMPApp competed with dATP for incorporation into nascent DNA and, since it lacked a 3' hydroxyl group, caused premature chain termination. The kinetic inhibition (Ki) constants and Ki(s) for human DNA polymerases are shown Table 25.

Table 25

Kinetic inhibition constants of PMPApp for human DNA polymerases

and HIV reverse transcriptase

Enzyme	Ki (μM)	Km dATP (μM)
HIV RT -DNA template	1.6	4.6
HIV RT -RNA template	0.02	0.05
alpha	5.2	2.7
beta	81.7	5.6
gamma	59.5	0.7

Comments: For PMPApp, the Ki value (5.2 μM) of α polymerase and the Km value (2.7 μM) are rather close. The closeness of these values suggests that the test compound may have a toxic effect on the normal human α polymerase.

Summary report: In vitro cytotoxicity of tenofovir in various human cell types-comparison with other NRTIs, April, 18, 2001, (C4331-00013)

Tenofovir only weakly inhibited the proliferation of liver-derived HepG2 cells and normal skeletal muscle cells with CC_{50} values > 500 μM, ZDV, ddC, ddI, d4T and abacavir all exhibited more pronounced cytotoxicity in these two cell types than tenofovir. No substantial effects on the expansion of erythroid and myeloid hematopoietic progenitor cells were observed in the presence of tenofovir at the concentrations substantially exceeding those needed for its antiviral activity in PBMCs (CC_{50} = 85 to > 200 μM vs EC_{50} = 0.2 μM). In contrast, ZDV, ddC and d4T interfered primarily with the growth of the erythroid progenitor linage with CC_{50} values ranging from 0.03 to 5 μM. Finally, tenofovir showed less effects on proliferation and viability of renal proximal tubule epithelial cells than cidofovir and adefovir. Conclusions: tenofovir exhibited weak cytotoxic effects in all cell types tested with substantially less cytotoxicity than the majority of NRTIs currently used for the treatment of HIV infection.

Appendix # 2

Synopsis of tenofovir DF and tenofovir acute animal toxicity studies.

Table 1
Summary of tenofovir DF acute toxicity studies in rats and dogs

Species	Dose level (mg/kg)	Deaths	Approx. NOAEL (mg/kg)	BSA equiv dose in man (mg/kg)	Major toxic signs
Rats, po,	160 500 1500	none	1500	14.6 g/day	none
Dogs, po	30 90 270	none	30	973 mg/day	renal cortical tubular basophilia & karyomegaly

BSA = Body surface area equivalent factor

Appendix # 3

Synopsis of tenofovir DF multiple dose oral toxicity studies in rats.

Table 2
Summary of the 13- and 42-week oral gavage toxicity study (with a 13-week recovery period) of bis-POC PMPA in albino rats

Dose levels & groups	Major drug-related findings
0 (controls), 20 rats/sex	Microscopically: slight renal tubular pigment accumulation at wk 43
Recovery, 5 rats/sex	None
30 (low) 20 rats/sex	Transient salivation. Microscopically: slight renal tubular karyomegaly and pigment accumulation at wk 4
Recovery, 5 rats/sex	None
100 (mid) 20 rats/sex	Salivation (mild. Increased RBC (♂ at wk 42). Decreased cholesterol (♂ at wk 26) Increases in urinary deoxypyridinoline (♂ wk 12 & ♀ wk 41). Microscopically: slight renal tubular karyomegaly and pigment accumulation (wk 43. Slight epithelial cell hypertrophy in the duodenum (wk 43)
Recovery, 5 rats/sex	Microscopically: slight renal tubular pigment accumulation.
300 (high) 20 rats/sex	Salivation (mild to moderate). Increases in RBC (♂ at wk 42 and platelet counts (♀ at wk 13/26)). Increases in ALT & AST, phosphorus and A/G ratios (♂ at wk 1/26/42). Decreases in cholesterol, total protein, globulins and triglycerides (♂ at wk 13/26/42); bicarbonate (♀ at wk 42). Decreases in cortical area, bone mineral content and density (♂) of femur metaphysis and mid femur regions. Increases in urinary deoxypyridinoline (♂ wk 12/42). Increases in osteocalcin (♂ at wk 42.
Recovery 5 rats/sex	Microscopically: slight to mild renal tubular karyomegaly (wk 14/43) and pigment accumulation (wk 43), slight gastritis (ulcerative) at wk 14, colitis (wk 43) and slight to mild typhlitis (wk 14/43). Slight duodenal epithelial hypertrophy and mild mucosal hyperplasia (wk 43).
	Microscopically: slight renal tubular Karyomegaly and pigment accumulation, mild typhlitis (wk 43).
1000 (very high) 20 rats/sex	Salivation (moderate to severe), decreased body weight gain and food consumption (♂). Increases in WBC, red cell distribution width, RBC, segmented neutrophil, platelet counts and decreases in hematocrit, hemoglobin, MCV (wk 13/26/42). Increases in ALT & AST, phosphorus, A/G ratios and creatinine (wk 13/26/42) and decreases in cholesterol, total protein, globulins and triglycerides (wk 13/26/42) and bicarbonate (♀, wk 42). Decreases in bone mineral content and density of the total slice and trabecular and cortical/subcortical areas of distal femur metaphysis and mid-femur diaphysis regions. Decreases in cortical thickness generally correlated with slight decreases in periosteal circumference and slight increases in endosteal circumference. Increases in urinary deoxypyridinoline, calcium and phosphorus (wk 12/25/42). Increases in PTH and osteocalcin (wk 14/43). Increases in adrenal weights. Microscopically (♂+♀): slight to mild renal tubular karyomegaly (wk 14/43) and slight to moderate pigment accumulation (wk 43). Slight to moderate typhlitis, mild jejunitis and gastritis (ulcerative and/or hyperkeratosis) at wk 14/43 and mild colitis, moderate duodenitis and mild ileitis at wk 4. Slight to mild duodenal (wk 14/43) and jejunal (wk 43) epithelial hypertrophy and slight to moderate duodenal mucosal hyperplasia (wk 43). Atrophy of the cecum (slight to moderate) and colon (slight to moderate, and slight villous atrophy of the ileum (wk 43).
Recovery 5 rats/sex	Microscopically: slight to mild renal tubular karyomegaly and slight pigment accumulation. Increases in PTH at wk 56 and osteocalcin at wk 49.

Appendix # 4

Synopsis of tenofovir DF multiple dose oral toxicity studies in animals and comparison with the clinical doses.

Table 1

Summary of tenofovir DF repeated oral toxicity studies in animals and comparison with the clinical doses (300 mg, AUC = 3.18 µg*hr/ml) tablets

Study	Dose levels (mg/kg/day	Major toxic signs, histopath. & laboratory findings	NOEL/NOAEL, mg/kg/day	BSA in humans
Rats, 2-week	30 100 300	None	300	2.9 g/day
Rats, 4-week	20 100 500	decreased kidney weights, decrease in RBC parameter	100	974 mg/day
Rats, 13-week	30 100 300 1000	histopath: changes in GI tract and kidney	100	974 mg/day
Rat, 42-week	30 100 300 1000	histopath: changes in GI tract and kidney; bone toxicity	<30	<292 mg/day

Dogs, 4-week	3	Increases in BUN; histopath: kidney-tubular degeneration, regeneration	3	97.2 mg/day
	10			
	30			
Dogs, 13-week	3	Chemistry changes: increase in AST, ALP; histopath:lesions in kidney	<3	<97.2 mg/day
	10			
	30			
Dogs, 42-week	3	Kidney, GI and bone toxicities	NOELs for bones: 10	973 mg/day
	10			
	30			

Table 2
Summary of estimated safety margin of GI, renal and bone toxicity in rats, dogs and monkeys

Target organ effect	Species	Study duration	NOEL,MEL mg/kg/day	AUCss µg*hr/ml	Margin relative to human AUCss
GI Toxicity	Rat	42-week	30,100	4,8	1, 3X
	Dog	42-week	30	30	10X
	Monkey	56-day	250	15	5X
Renal toxicity	Rat	42-week	300,1000	18,65	6,20X
	Dog	42-week	3,10	2,7	1,2X
	Monkey	56-day	250	15	5X
Reduced serum phosphate	Rat	42-week	1000	65	20X
	Dog	42-week	30	30	10X
	Monkey	56-day	<30	4	<1X
Bone mineral loss	Rat	42-week	100,300	8,18	3,6X
	Dog	42-week	10,30	7,30	2,10X
	Monkey	56-day	ND	NA	ND

Appendix # 5

Synopsis of tenofovir multiple dose sc or iv toxicity studies in SIV-infected and healthy rhesus monkeys: bone toxicity

Table 1
Summary of Martin study in SIV-infected rhesus monkeys

Group	N	PMPA	Time of start of	Length	Deaths/days	No. of

		mg/kg/day sc	dosing post SIV infection	treatment		monkeys with bone lesions
1	5	0	24 hr	28 days	4, 182-276	0/5
2	5	30	24 hr	28 days	3, 182-376	0/5
3	5	30	7 days	28 days	2, 239-284	0/5
4	5	30	14 days	28 days	4, 246-323	0/5
5	5	30	14 days	434	0,	5/5*

* Animals showed bone lesions after 12 months of daily sc doses of 30 PMPA mg/kg/day

Table 2

PMPA pharmacokinetics in SIV infected adult rhesus monkeys (Martin & Tsai)

Dose	Duration	Cmax (µg/ml)	AUC µg*hr/ml	T½ (hr)	CL/F ml/hr/kg	Bone lesions
30 sc	14 mont	47.4	144	6.04	209	yes
30 sc	14 mont	52.9	145	6.72	207	yes
30 sc	14 mont	33.7	97.9	3.94	306	yes
30 sc	14 mont	39.8	154	5.97	195	yes
30 sc	14 mont	53.5	240	6.27	125	yes
10* iv	single	27.7	14.7	10.6	710	no

* Tsai study performed at Univ of Washington, Naive animals administered a single iv dose of 10 mg/kg PMPA

Human AUCss (3.18 µg*hr/ml) following a 300 mg/day dose

Table 3

Selected clinical pathology and pharmacokinetics of PMPA (10 mg/kg/day for 27-30 months in healthy and SIV infected rhesus monkeys

Animal sex/SIV status	Age/ treatment period	Serum Ph mg/dl	ALP U/L	Cmax µg/ml	$AUC_{...}$ µg*hr/ml	Glycosuria/proteinuria
♂ healthy	2 days, 30 months	6.2-7.7	673-1573	17.4	18	negative
♀ healthy	1 day,30 months	5.8-8	708-1408	10	10	negative
♀ infected	23 days,27 months	4.2-7.4	580-788	10.4	13	negative

Human AUCss (3.18 µg*hr/ml) following a 300 mg/day dose

Appendix # 6

Synopsis of single oral dose pharmacokinetic parameters of tenofovir DF in animals

Table 1
Pharmacokinetic parameters of tenofovir DF following oral administration of single doses in animals

Species	Dose (mg/kg)	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)	Cl/F l/hr/kg	F (%)
Rat 97-TOX-002-PK	30	2.87	0.46	0.38	6.21	4.7	27.9
	100	6.64	1.17	0.75	10.7	6.8	19.4
	300	17.7	1.51	0.75	17.3	7.6	17.2
	1000	43.5	2.71	0.75	29.6	10.3	12.7
Beagle dog 97-TOX-001-PK	3	nc	0.21	0.625	nc	nc	nc
	10	nc	0.66	0.5	nc	nc	nc
	30	9.57	2.95	0.625	22.1	1.3	22.2
Rhesus monkey	5	0.725	0.11	0.83	8.23	3.2	32.4
	50	6.38	1.15	1	8.54	3.8	23.7
	250	14.8	1.68	1.08	8.41	6.5	17

Appendix # 7

Comparison of tenofovir pharmacokinetic parameters between species following single oral dose administration of tenofovir DF

Table 1

Comparison of tenofovir pharmacokinetic parameters between species following single oral dose administration of tenofovir DF

Species	Dose (mg/kg)	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)	Cl/F l/hr/kg	F (%)
Rat	40	2.55	1.06	0.25	6.88	7	18.6
	400	16.3	3.1	0.5	19.7	11	11.9
Dog	5	3.14	0.44	1.25	79.9	0.75	31
	30	24.4	4.09	0.875	47.4	0.58	43.8
Rhesus monkey	5	0.72	0.11	0.83	9.2	3.2	32.3
	50	6.4	1.15	1	9.4	3.8	23.7
	250	19	2.15	0.58	8.9	6.7	14.5
Human	4.12	3.2	0.36	2.1	13	0.6	39.7
	7.66	5	0.61	1.4	12.7	0.7	34.4

Appendix # 8

Comparison of tenofovir pharmacokinetic parameters between species following iv administration of tenofovir

Table 1
Comparison of tenofovir pharmacokinetic parameters between species following iv administration of tenofovir

Species	Dose (mg/kg)	Pharmacokinetic parameters				
		AUC (µg*hr/ml	Cmax (µg/ml)	T½ (hr)	Cl l/hr/kg	Vss l/kg
Rat	10	5.86	22	4	1.7	2.8
	50	53.7	162	5.4	0.93	1.1
Dog	1	4.5	3	45.3	0.22	3
	10	41.2	31	38.6	0.24	2.5
Rhesus monkey	5	5.14	13.8	7	1	1.2
	30	38.4	79	10.6	0.88	0.96
Human	1	4.4	2.7	5.3	0.23	0.76
	3	15.2	8.5	7.8	0.22	0.97

Appendix # 9

Synopsis of multiple oral dose tenofovir DF pharmacokinetics in

animals

Table 1

Mean pharmacokinetic parameters of tenofovir in plasma following repeated oral administration of tenofovir to rats

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
30	day 1	2.87	0.46	0.38	6.21
	week 13	2.65	0.56	0.75	4.93
	week 26	3.93	0.66	0.5	8.3
	week 42	3.78	0.74	0.5	7.24
100	day 1	6.64	1.17	0.75	10.7
	week 13	7	1.4	0.75	7.7
	week 26	6.89	1.36	0.5	8.9
	week 42	8.34	1.86	1	9.34
300	day 1	17.7	1.51	0.75	17.3
	week 13	18.4	2.86	2.5	7.21
	week 26	13.8	2.32	1	11
	week 42	17.6	2.18	1	9.1
1000	day 1	43.5	2.71	0.75	29.6
	week 13	34.1	4.52	0.5	25.6
	week 26	42.8	5.82	1	12.1
	week 42	64.85	6.57	1.5	9.66

Table 2

Mean pharmacokinetic parameters of tenofovir in plasma following repeated oral administration of tenofovir to male and female dogs for 42 weeks

Dose (mg/kg/day)	Period	Pharmacokinetic parameters			
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)
3	day 1	nd	0.25	0.5	-
	week 13	nd	0.22	0.75	-
	week 26	nd	0.26	1	-
	week 42	2.12	0.29	1	34.4
10	day 1	nd	0.66	0.5	-
	week 13	5.73	1.37	0.5	27.3
	week 26	5.92	1.01	0.75	40.6
	week 42	6.63	1.4	0.5	37.9
30	day 1	11.5	2.46	0.5	18.8
	week 13	29.3	7.36	0.75	31.4
	week 26	29.8	8.15	0.75	22.8
	week 42	29	7.52	1	28.2

Appendix # 10

Synopsis of single and multiple oral dose tenofovir DF pharmacokinetics in HIV-infected patients

Table 1

Pharmacokinetics of tenofovir following single and multiple doses

of 300 mg and 600 mg tenofovir DF to HIV-infected patients (study # 901)

Dose (mg/day)	Day	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)	CL/F ml/hr/kg	F (%)
300	1	2.09	0.24	0.8	11.9	910	24.9
	8	3.17	0.37	2	11.7	584	38.8
	15	-	0.30	3	13.7	521	-
	35	-	0.32	2.3	14.4	510	-
600	1	3.37	0.62	1	13	1083	20.9
	8	4.38	0.57	1.5	11.9	671	33.8
	15	-	0.63	2.5	12.1	683	-
	35	-	0.5	2	15.4	641	-