

(mg/kg)							
Oral 30.0	0.96 ± 0.8	5	149	26.2	5.6	126	11
IV 30.0	31.3 ± 4.9	10	156	27	6.2	1098	100

23. Pharmacokinetics, Mass Balance and Tissue Distribution of Radiolabelled ¹⁴C-PMEA, Lot # NB 316-95-02 and NB 376-14-9-01, August 20, 1992, (830-GS-001-92)

Two groups of adult male and female rats (strain: Sprague Dawley; weight: 258 - 324 g; 4 animals/sex/group) were given a single 10 mg/kg intravenous dose of ¹⁴C-PMEA (approximately 50 - 100 µCi). Blood samples (150 - 200 µl) were obtained from one group of rats by retro-orbital puncture prior to dosing and serially for 24 hr post-dose (Group 1). Urine and feces were cage-collected prior to dosing and for 6 hr post-dose from the other group of rats (group 2). Six hr post-dose, rats in Group 2 were sacrificed by CO₂ inhalation and specified tissues/organs were obtained. Radioactivity content was determined in all the samples by scintillation counting following sample oxidation. The objective of this study was to characterize the pharmacokinetics, mass balance, tissue distribution and routes of elimination of ¹⁴C-PMEA. In-Life Observation: One animal died immediately after dosing. Complete necropsy of the animal revealed death was attributable to an air embolism from the injection and was not considered attributable to test article administration; this animal was replaced to provide full study groups for the treatments. Five animals in Group 1 and seven in Group 2 exhibited a dark red color around the genital area and in the 0 - 6 hr urine, respectively. This was attributable to test article.

Plasma Radioactivity Concentrations and Pharmacokinetic parameters From Group 1 Rats: Mean plasma radioactivity concentrations versus time are summarized in Table 17. Concentrations decreased rapidly during the first 2-hr followed by a slower elimination phase from 2 to 6 hr post-dose. Concentrations at 24 hr post-dose could not be detected in all animals. Pharmacokinetic parameter values are shown in Table 18.

Table 17

¹⁴C Concentrations (µg.eq/ml) in Plasma of Rats After Single 10 mg/kg Intravenous Dose of ¹⁴C-PMEA

Statistical Parameters	Time (Hour)								
	0.03	0.08	0.25	0.50	1.0	2.0	4.0	6.0	24.0
	Rat Plasma ¹⁴ C Concentrations (µg.eq/ml)								
Mean	49.5	32.7	16.7	8.4	2.7	0.6	0.2	0.1	0.0
S.D.	8.4	4.7	3.9	2.9	1.2	0.2	0.07	0.04	0.0
% Coefficient Variation	17.0	14.5	23.3	34.4	44.5	40.1	39.6	40.2	0.0

Table 18

Pharmacokinetic Parameters For Radioactivity in Rat Plasma After Single 10 mg/kg Intravenous Dose of ¹⁴C-PMEA

Statistical Parameters	C ₀ (µg/ml)	t _{1/2} (hr)	Cl _B (ml/min/kg)	Cl _R (ml/min/kg)	Cl _T (ml/min/kg)	V _D (l/kg)
Mean	0.22	1.81	16.7	10.0	6.32	3.68
S.D.	0.05	0.24	3.7	2.8	-	0.50
% Coefficient of Variation	22.0	13.2	22.2	28.2	-	30.6

Tissue Recovery of Radioactivity Six-Hour Post-Dose From Group 2 Rats: Mean tissue radioactivity concentrations are summarized in Table 19. Highest concentrations were found in kidney followed by cecum, small intestine, liver and large intestine. All other tissue concentrations averaged less than 0.5 µg.eq/g. The highly perfused lung tissue averaged 0.37 µg.eq/g; tissues exhibiting the lowest levels of radioactivity were brain, testes and skeletal muscle. Kidney radioactivity concentration of 18.7 µg.eq/g was from 3 to 934 times that of any other tissue concentration. However, the highest radioactivity concentration (% of dose) was found in the residual carcass which averaged 2% of the administered dose. Total radioactivity recovery in all

tissues combined averaged 6.72% of the administered dose at 6 hours.

Table 19
¹⁴C Concentrations (µg.eq/g) in Rat Tissues Six-Hour After Single 10 mg/kg Intravenous Dose of ¹⁴C-PMEA

Tissues	Tissue Recovery			Recovery (% of Dose)	
	Mean (µg.eq/g)	S.D.	Relative S.D.	Mean	S.D.
Adrenal	0.41	0.32	77.8	0.0	0.0
Brain	0.02	0.00	42.5	0.0	0.0
Cecum	7.03	6.1	86.7	0.8	0.6
Heart	0.16	0.03	20.5	0.01	0.0
Kidney	18.7	2.67	14.3	1.7	0.4
Large Intestine	1.8	1.17	65.9	0.14	0.1
Liver	3.3	1.1	33.9	1.13	0.4
Lung	0.37	0.08	22.6	0.02	0.0
Mesenteric Lymph Node	0.33	0.1	28.8	0.0	0.0
Ovaries/Uterus	0.13	0.01	8.6	0.0	0.0
Pancreas	0.38	0.09	24.6	0.01	0.0
Residual Carcass	0.24	0.05	20.0	2.0	0.4
Skeletal Muscle	0.05	0.02	30.9	0.0	0.0
Small Intestine	3.44	3.1	89.4	0.9	0.7
Stomach	0.13	0.03	23.0	0.01	0.0
Testes	0.05	0.01	30.9	0.0	0.0
Thymus	0.31	0.06	19.8	0.01	0.0
TOTAL	36.8	-	-	6.72	-

Urinary, Fecal and Tissue Recovery of Radioactivity Six-Hour Post-Dose From Group 2 Rats: Mean radioactivity recoveries in urine, feces and tissues are summarized in Table 20. The urinary recovery of PMEA as a percentage of the administered dose averaged 62.63%; however, the total cumulative radioactivity recovered was about 83%. Fecal recovery of radioactivity accounted to about 0.32% of the recovered radioactivity. Approximately 70% of the total dose was recovered in urine, feces and specified tissues; on the other hand, the total radioactivity recovery was about 90%.

Table 20

¹⁴C Recovery (% of Dose) in Urine, Feces and Tissues of Rats After Single 10 mg/kg Intravenous Dose of ¹⁴C-PMEA

Statistical Parameters	% of Dose in Urine (U)	% of Dose in Feces (F)	% of Dose in Tissues (T)	Total % of Dose in U,F,T	Of the total radioactivity recovered, % in U	Of the total radioactivity recovered, % in F
Mean	62.63	0.13	6.72	69.4	82.73	0.32
S.D.	34.2	0.25	1.6	33.8	22.83	0.72
% Coefficient of Variation	54.6	197.1	23.8	48.7	27.6	44.4

Comments: The recoveries of PMEA either as 'cold compound' or as ¹⁴C-PMEA were highly variable as indicated by S.D. values; and/or the assay methods utilized in analysis of both species of PMEA are not precise as indicated by the coefficient of variation values (24 - 197%). Despite these deficiencies in the experimental designs, nonetheless, the major route of elimination of PMEA is by urinary elimination as most of the dose recovered unchanged in the urine. Among all the organs tested for PMEA deposits, the kidney may function as the depot for the drug. Though the highest percentage of the drug was found in the residual carcass most of it may be in the skin as suggested by the revealed toxicity to this organ in animal toxicity studies. The increased recovery of the drug by ¹⁴C measurement method (90%) over the parent drug analysis method by — (70%) may be due to the fact that the ¹⁴C measurement method is more sensitive than — alternatively, the ¹⁴C measurement method may be picking up ¹⁴C-metabolite(s) of the drug thus contributing to the higher recovery as compared to the one attained by — method.

In the mass balance and tissue distribution of ¹⁴C-PMEA following single dose iv administration to rats, the total cumulative recovery of ¹⁴C-PMEA is about 90%; ideally, it should be 100%. The sponsor has not measured exhaled ¹⁴CO₂ which must be taken into consideration in a mass balance experiment.

24. Bioequivalency study of ¹⁴C labeled GS0393 after administration to Cynomolgus Monkeys, Lot # NB316-95-50, December 17, 1992, (2-K03/—-GSI-K03-92-111)*

The study consisted of one group of two male and female cynomolgus monkeys. Each animal was treated a total of four times with the test article according to a design described in Table 21. The objective of the study was to determine the

bioavailability of ¹⁴C-GS0393 following a single iv, sc, im or po administration to cynomolgus monkeys.

Table 21
Study Design of the Bioequivalency Study of ¹⁴C-GS0393 in Cynomolgus Monkeys

Group #	No. Animals	Dose (mg/kg)	Route	Treatment Day	Blood Collection Time (Post-Dose)
1	2 males and 2 females	10	iv	1	5, 15, 30 min, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96 hr
		10	sc	15	5, 15, 30 min, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96 hr
		10	im	29	5, 15, 30 min, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96 hr
		10	po	43	5, 15, 30 min, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96 hr

All animals survived until the scheduled termination of the study. Clinical observations consisted of bruising in the inguinal areas of all monkeys. Other incidental observations included decreased feces production in three of four animals, and pale mucous membranes, scratches to both eyes and a clear discharge in a female monkey.

Plasma ¹⁴C data appeared to demonstrate a bi-exponential decline in ¹⁴C plasma activity for the iv route. Available pharmacokinetic parameters for all four dose administration routes are shown in Table 22.

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Table 22
Pharmacokinetic Parameters For ¹⁴C-GS0393 Following the Administration to Cynomolgus Monkeys

Route of Administration

Parameters	IV	SC	IM	PO
AUC (CPM*hr/ml)	50737	-	-	-
t _{1/2} α (hr)	0.2	-	-	-
t _{1/2} β (hr)	0.98	-	-	-
V _{ss} (ml)	1817	-	-	-
F (%)	100	113	112	3.6

Comments: The method utilized for PMEA determination in plasma for the monkeys is based upon the measurement of ¹⁴C activity (CPMs) and does not necessarily measures the parent drug. ¹⁴C-GS0393 was 100% bioavailable when administered via the sc or im dose routes to cynomolgus monkeys, but demonstrated very poor absorption (3.6%) via the oral route.

25. Pharmacokinetics of adefovir in a ninety-one day repeated dose intravenous or subcutaneous toxicity study of adefovir administered to cynomolgus monkeys, February 23, 1999, (93-TOX-0393-001-PK)

Groups of male and female cynomolgus monkeys (6 animals/sex/group) were administered PMEA by a subcutaneous or intravenous injection at 0 (vehicle control; both sc and iv), 3 (low; iv), 8 (mid; iv)/(mid; sc) or 20 mg/kg/day (high; iv) in sterile saline for a period of 91 consecutive days. Blood samples were obtained at intervals over 12 hr post dose on days 1, 22, 50 and 78. Concentrations of adefovir in plasma were determined using a validated method. Results: mean pharmacokinetic parameters for adefovir in male and female monkeys are shown in Tables 23 and 24, respectively. Repeated administration of iv or sc adefovir over the range of 3 to 20 mg/kg/day did not lead to significant changes in AUC values or other pharmacokinetic parameters. The estimated sc bioavailability of adefovir in male monkeys was 136% on day 1 and 104% on day 78. The estimated sc bioavailability of adefovir in female monkeys was 123% on day 1 and 122% on day 78.

Table 23

Mean pharmacokinetic parameters for adefovir following repeated daily iv or sc administration to male monkeys

Dosage (mg/kg/day)	C ₀ (µg/ml)			AUC _{0-∞} (µg/ml)			T _{1/2} (hr)			Cl (l/hr/kg)		
	drug days			drug days			drug days			drug days		
	1	50	78	1	50	78	1	50	78	1	50	78

3, iv	7.9	13.4	13.6	9.7	9	10.4	2.7	23	1.5	0.4	0.36	0.3
8, iv	20	56.5	28.3	14.6	35	20	1.2	1.9	2	0.6	0.3	0.4
20, iv	62	64	82	53.2	48.2	62	2.2	2.4	2	0.4	0.4	0.3
8, sc	14	14.4	14	20	21.2	20.4	1.2	2	2.2	na	na	na

Table 24

Mean pharmacokinetic parameters for adefovir following repeated daily iv or sc administration to female monkeys

Dosage (mg/kg/day)	C0 (µg/ml)			AUC ₀₋₂₄ (µg/ml)			T _{1/2} (hr)			Cl (l/hr/kg)		
	drug days			drug days			drug days			drug days		
	1	50	78	1	50	78	1	50	78	1	50	78
3, iv	7.8	11	9.4	4.4	5.8	4.6	0.95	1.3	0.7	0.7	0.5	0.7
8, iv	21	25	23	12.2	14.6	12.4	1.5	1	0.9	0.7	0.6	0.8
20, iv	64	65	59	38	44	41	1	2.2	2	0.6	0.5	0.6
8, sc	11	12	11	15	17	15	2.4	2.2	1.2	na	na	na

26. Pharmacokinetics of adefovir in pregnant rats and rabbits after iv administration of adefovir or oral administration of adefovir dipivoxil, 27 March, 1998 (96-TOX-0840-002-PK)

Groups of presumed pregnant rats (16/group) received adefovir dipivoxil via oral gavage at dose levels of 6.25 or 25 mg/kg/day and plasma adefovir levels were determined after a single dose or after 10 consecutive days of dosing. In addition, plasma adefovir levels were determined following either a single iv dose or 10 consecutive daily doses of adefovir at 2.5 or 10 mg/kg/day. Rabbits (3/group) received a 20 mg/kg/day dose of adefovir dipivoxil and plasma levels were either obtained after a single dose or after 13 consecutive days of dosing. Results: are shown in Tables 25 and 26. No apparent changes were observed in the pharmacokinetics of adefovir in pregnant rats following 10 consecutive daily iv doses of adefovir (2.5 mg/kg/day). Following iv administration of 10 mg/kg/day adefovir to pregnant rats, exposure to adefovir was 44% greater and clearance was decreased 30% following the 10th dose compared to the first dose.

Table 25

Pharmacokinetic parameters for adefovir in plasma following iv administration of adefovir to pregnant rats

Dosage (mg/kg/day)	C ₀ (µg/ml)		AUC ₀₋₂₄ (µg*hr/ml)		T _{1/2} (hr)		Cl (l/hr/kg)	
	drug days		drug days		drug days		drug days	
	1	10	1	10	1	10	1	10
2.5	13.4	9.8	2.58	2.91	-	-	0.96	0.86
10	31.6	35.3	7.98	11.5	1.5	2	1.25	0.87

Table 26

Pharmacokinetic parameters for oral administration of adefovir dipivoxil to pregnant rats and rabbits

Dosage (mg/kg/day)	C _{max} (µg/ml)		AUC ₀₋₂₄ (µg*hr/ml)		T _{max} (hr)		F (%)	
	drug days		drug days		drug days		drug days	
	1	10	1	10	1	10	1	10
Rats, 6.25	0.26	0.37	0.82	1.33	0.5	0.5	29.6	48.1
Rats, 25	1.58	1.2	4.7	3.7	1	1	43.4	33.7
Rabbits, 25	2.1	2.51	8.5	12.1	0.5	1.5	-	-

27. Bioavailability of PMEA from two clinical formulations of the Prodrug bis-POMPMEA in beagle dogs, Gilead Science, Foster City, CA, March 7, 1995, (94-DDM-0840-002)

The oral bioavailability of PMEA was determined for 2 formulations of bis-POMPMEA in beagle dogs. The formulations of bis-POMPMEA (20 mg/kg) examined included a suspension of granules in concentrated grape juice and tablets prepared from the same granulation. The suspension was evaluated in fasted animals. The tablets were evaluated in both fasted and fed states and fasted dogs pretreated with pentagastrin to mimic the pH of the human intestine. Concentrations of PMEA observed following the administrations were compared to data for iv PMEA (10 mg/kg) in the same animal. Results: are shown in Table 27. Conclusions: these data indicated that oral bioavailability of PMEA from adefovir dipivoxil in dogs appeared independent of formulation, food or pH of the gastrointestinal tract.

Table 27

Bioavailability of PMEA from adefovir dipivoxil in beagle dogs

Formulations	Feeding state	Tmax (hr)	Cmax (µg/ml)	F (%)
Suspension of granules in grape juice	fasted	1.9	1.5	35
Tablets	fasted	1.4	1.6	34.7
Tablets	fed	1.2	2	37.2
Tablets	fasted, pentagastrin pretreated	1.7	2.2	44.9

28. Bioavailability of PMEA from tablet formulations of the Prodrug bis-POMPMEA in beagle dogs, Gilead Science, Foster City, CA, March 26, 1998, (94-DDM-0840-004)

The oral bioavailability of PMEA from a new clinical batch of bis-POMPMEA (batch # 840B94-01) was evaluated in five pentagastrin pretreated, fasted beagle dogs. The tablets were administered at a dose level of two tablets (125 mg adefovir dipivoxil per tablet) per animal by gavage, followed by 30 ml water. Results: are shown in Table 28. Conclusions: the oral bioavailability of PMEA from the new clinical batch of adefovir dipivoxil was not significantly different from that seen with batch # 840H94-1.

Table 28

Bioavailability of PMEA from adefovir dipivoxil (new clinical batch # 840B94-1) in beagle dogs

Batch #	Tmax (hr)	Cmax (µg/ml)	F (%)
840B94-01	1.7	2.2	44.9
840H94-01 (old batch)	2.1	2.6	43.6

29. Bioavailability of PMEA from various formulations of the Prodrug bis-POMPMEA in monkeys, Gilead Science, Foster City, CA, May 13, 1994, (DDM-JPS-020394)

The oral bioavailability of PMEA was determined from three PMEA prodrugs following oral administration to cynomolgus monkeys. The

three prodrugs evaluated were bis-POMPMEA, bis-POEPMEA and bis-PMEA. All formulations were administered at 10 mg-equivalent of PMEA per kg. Concentrations of PMEA observed following the administrations were compared to data for iv PMEA (10 mg/kg) in the same animal. Results: are shown in Table 29.

Table 29

Bioavailability of PMEA from various formulations of PMEA prodrugs in cynomolgus monkeys

Prodrug	Formulation	Tmax (hr)	Cmax (µg/ml)	F (%)
bis-POMPMEA	granule/grape juice	0.8	0.36	31.9
	tablets	7.4	0.19	14.3
	PEG 400	2	0.74	32.7
bis-POEMEA	PEG 400	0.8	0.36	17.4
bis-PMEA	PEG 400	0.9	0.21	17.5
	50% PEG 400	3.5	0.86	40.2

30. Bioavailability of PMEA from the Prodrug bis-POMPMEA in monkeys, Gilead Science, Foster City, CA, May 13, 1994, (DDM-JPS-020394)

The oral bioavailability of PMEA was determined from three PMEA prodrugs following oral administration to cynomolgus monkeys. The three prodrugs evaluated were bis-POMPMEA, bis-POEPMEA and bis-PMEA. All formulations were administered at 10 mg-equivalent of PMEA per kg. Concentrations of PMEA observed following the administrations were compared to data for iv PMEA (10 mg/kg) in the same animal. Results: are shown in Table 30.

Table 30

Bioavailability of PMEA from various formulations of PMEA prodrugs in cynomolgus monkeys

Prodrug	Formulation	Tmax (hr)	Cmax (µg/ml)	F (%)
bis-POMPMEA	granule/grape juice	0.8	0.36	31.9
	tablets	7.4	0.19	14.3
	PEG 400	2	0.74	32.7
bis-POEMEA	PEG 400	0.8	0.36	17.4

bis-PMEA	PEG 400	0.9	0.21	17.5
	5% PEG 400	3.5	0.86	40.2

31. Bioavailability of PMEA from the Prodrug bis-POMPMEA in monkeys: bis-POMPMEA Sulfate, and a clinical tablet formulation of bis-POMPMEA, Gilead Science, Foster City, CA, March 6, 1998, (94-DDM-0840-001B)

The two prodrugs: bis-POMPMEA Sulfate (suspension in 5% PVP K-15 polyvinylpyrrolidone) and a clinical tablet formulation were evaluated following oral administration to cynomolgus monkeys. Results: are shown in Table 31.

Table 31

Oral bioavailability of PMEA from two different prodrug formulations in cynomolgus monkeys

Prodrug	Formulation	Tmax (hr)	Cmax (µg/ml)	F (%)
bis-POMPMEA Sulfate	Suspension in 5% PVP K-15	2.2	0.56	25.3
bis-POMPMEA (GS0840)	Clinical tablets	9.6	0.55	27

32. Urinary excretion of ¹⁴C-adefovir following intravenous administration to Sprague-Dawley rats, 19 March, 1998 (94-DDM-0393-004)

Four male rats received a single iv injection of ¹⁴C-adefovir (10 mg/kg; 400 µCi/kg) to evaluate the urinary excretion. Urinary samples were collected over predetermined intervals for a 7 day period. Results: The mean recovery of the administered dose in the urine was 83.1% by 24 hr and 85% by 7 days post dose. The mean terminal half-life for urinary elimination was 24.3 hr. Conclusions: these results indicated that the major route of elimination of adefovir is urinary excretion.

33. Toxicokinetic study of adefovir dipivoxil in presumed pregnant rats and rabbits, Lot # 01-167-DK, Gilead Science, Inc., Foster City, CA, April 16, 1997, (96-TOX-0840-002/-GSI-3D1096-225)

This study consisted of eight groups of presumed-pregnant female rats (Crl:CD BR VAF/Plus) and two groups of presumed-pregnant female New Zealand White rabbits receiving iv adefovir or oral gavage adefovir dipivoxil according to a study design shown in Table 32. Blood samples were collected at protocol specified timepoints and were analyzed by a validated — method.

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Table 32
Study design

Group #	Number of females		Treatment administration			Euthanasia
	Rats	Rabbits	Test article	Dose (mg/kg/day)	Dosing regimen	
1	16	0	Adefovir iv bolus	2.5	once on day 6 of gestation	day 1
2	16	0		2.5	once daily for 10 days; days 6-15 of gestation	day 10
3	16	0		10	once on day 6 of gestation	day 1
4	16	0		10	once daily for 10 days; day 6-15 of gestation	day 10
5	16	0	Adefovir dipivoxil oral gavage	6.25	once on day 6 of gestation	day 1
6	16	0		6.25	once daily for 10 days; days 6-15 of gestation	day 10
7	16	0		25	once on day 6 of gestation	day 1
8	16	0		25	once daily for 10 days; days 6-15 of gestation	day 10
9	0	3		20	once on day 6 of gestation	day 1
10	0	3		20	once daily for 13 days; days 6-18 of gestation	day 13

Results: administration of adefovir (2.5 and 10 mg/kg/day) and adefovir dipivoxil (6.25 and 25 mg/kg/day) by iv injection or oral gavage, respectively, for up to ten consecutive days during the period of organogenesis was adequately tolerated in presumed pregnant rats. Likewise, oral gavage administration of adefovir

dipivoxil (20 mg/kg/day) was adequately tolerated following up to 13 daily administrations to presumed pregnant rabbits during the period of organogenesis. This was supported by the absence of any test article-related abnormal observations or body weight changes during the study period. The observed pharmacokinetic parameters are shown in Table 33.

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Table 33

Mean pharmacokinetic parameters of adefovir and dipivoxil adefovir in presumed pregnant female rats and rabbits

Group #	Number of females		Treatment administration		PK Parameters	
	Rats	Rabbits	Test article	Dose (mg/kg/day)	Cmax (µg/ml)	Tmax (min)
1	16	0	Adefovir iv bolus	2.5	day 1: 8.06	5
2	16	0		2.5	day 10: 7.65	5
3	16	0		10	day 1: 23.5	5
4	16	0		10	day 10: 30.9	5
5	16	0	Adefovir dipivoxil oral gavage	6.25	day 1: 0.262	30
6	16	0		6.25	day 10: 0.374	30
7	16	0		25	day 1: 1.58	60
8	16	0		25	day 10: 1.2	60
9	0	3		20	day 1: 2.1	30
10	0	3		20	day 13: 2.51	30

IV. GENERAL TOXICOLOGY:

General toxicology studies summary: The studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practice Regulations.

- Lot # JM-614-5,
January 6, 1994, 6511-101/93-TOX-0840-001)*
11. A 6-Month chronic oral toxicity study of bis-POMPMEA in Rats, Lot # 808-GS-93, 6511-101/93-TOX-0840-001 May 2, 1996, (94-TOX-0840-003\Project No. 86539)*
 12. A 4-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-5, 6511-100/93-TOX-0840-002)* January 5, 1994, 6511-100/93-TOX-0840-002)*
 13. A 13-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-70-11, 6511-103/94-TOX-0840-001)* September 1, 1994, 6511-103/94-TOX-0840-001)*
 14. A 52-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # TX840-96-01, 00019/95-TOX-0840-006)* February 5, 1998, (T0840-00019/95-TOX-0840-006)*
 15. Serum Carnitine Levels in Monkeys Administered Adefovir dipivoxil for 13 and 52 Weeks in Toxicity Studies (TOX-99-0840-001)

Multiple dose toxicology-Adefovir

16. Intravenous Multiple-Dose (x5) Range Study in Rats, 41671 (HPMPC), Batch # 26870-064 and 40085 (PMEA) Phosphonates, Batch # 26870-059, 20887/88032)* October 3, 1988, 20887/88032)*
17. Fourteen day repeated dose oral toxicity study of PMPA and PMEA in Sprague-Dawley rats, Lot # 891-161-32, Gilead Science, Inc., Foster City, CA, November 30, 1995, (95-TOX-1278-001)*
18. Multiple-Dose Intravenous Pilot Study in Rats, 41671 (HPMPC), Lot # 26870-64 and 40085 (PMEA), Lot # 26870-059, 20971/88072)* January, 1989, 20971/88072)*
19. One-Month Intravenous Toxicity Study in Rats, 40085 (PMEA), Identification # 27337-11 and 27337-15, 21489/124004)* January 24, 1990, 21489/124004)*

20. Thirty-day subcutaneous toxicity study of GS-0393 in rats, Lot # 1965-BL-1,
March 3, 1993, (UIC/ 109)*
21. Multiple-Dose Intravenous Pilot Study in Monkeys,
41671 HPMPC, Lot # 26870-64 and 40085 PMEA, Lot #
26870-059, January,
1989, (20954/88064)
22. Fourteen day repeated dose oral toxicity study and
pharmacokinetics of GS0393 administered via gavage
Cynomolgus Monkeys, Lot # 1965-BL-1,
January 8, 1993, (2-
M52/ -GSI-M52-92-146)*
23. One-Month Intravenous Toxicity Study in Cynomolgus
Monkeys, 40085 (PMEA), Identification # 27337-21,
December 28, 1989,
-21456, -124003)*
24. Thirty day repeated dose subcutaneous toxicity study
and pharmacokinetics of GS0393 administered to
Cynomolgus Monkeys, Lot # 1965-BL-2,
April 2, 1993, (2-M-
58)*
25. A ninety-one day repeated dose intravenous or
subcutaneous (2-M58/ML-GSI-M58-93-28) toxicity study and
pharmacokinetics of PMEA administered to cynomolgus
monkeys, Lot # 393B93-01,
June 29, 1994, (93-TOX-0393-001/ 2-
Q58)*

Review of general toxicology studies:

Single dose toxicology-Adefovir dipivoxil

1. A Single-Dose Oral Toxicity Study of bis-POMPMEA in Rats, Lot
not given, January 10, 1994,
(6511-102/93-TOX-0840-003)

Groups of male and female rats (weight: 146-221 g; age: 43 days; strain: Crl:CD BR VAF/Plus; 10 animals/sex/group) were administered a single oral gavage dose of bis-POMPMEA at dose levels of 0 (vehicle control), 24, 75 or 225 mg/kg. All the animals were observed for 17 days for signs of toxicity and changes in general health and behavior. Approximately 24 hr after the treatment, animals (225 mg/kg) had a lower urine pH and

slightly higher ALT and potassium. At the day 2 sacrifice (5 animals/sex/group), the stomach, duodenum, jejunum and ileum of animals (225 mg/kg) exhibited minimal to moderate individual epithelial cell necrosis, and the epithelial cells of the duodenal crypts showed karyomegaly. None of the aforementioned changes were seen on the day 17 sacrifice.

Comments: A single oral gavage dose of 75 mg/kg bis-POMPMEA in rats may be considered a NOEL. Based on an equivalent body surface area factor, an equivalent dose in humans would be approximately 12.17 mg/kg.

**2. Acute Intravenous Toxicologic Studies in Mice and Rats, — .
40085 (PMEA), Lot # 26807-087, _____,
N.Y., June 30, 1989, (_____ 21062/89020, 89022)***

Five groups of male and female Sprague-Dawley (Cr1:CD (SD) BR) rats (weight: 198 - 224.4 g male and 166.5 - 185.1 g female; age: 7 weeks, 5 animals/sex/group) were given PMEA a single intravenous doses of 100, 250, 500 and 1000 mg/kg, and three groups of male and female (CD-1 (Cr1:CD-1(ICR)BR) mice (weight: 20.1 - 29.5 g; age: 5.5 weeks, 5 animals/sex/group) were administered a single intravenous doses of 250 or 500 mg/kg. Control groups (5 male and 5 female) of rats and mice received 0.9% sodium chloride under identical experimental conditions. The objective of the study was to investigate the acute intravenous toxicity of PMEA in mice and rats. All the animals were observed for 14 days for signs of toxicity and changes in general health and behavior. Rats: having shown first decreased activity, ataxia and lethargy, all animals (500 and 1000 mg/kg) died within 24 hr after dose administration. Both male and female rats (250 mg/kg) exhibited decreased activity and female rats were slightly lethargic. Fifty to 75 min after dosing, all rats (100 mg/kg) appeared to be normal clinically throughout the observation period. All rats (100 and 250 mg/kg) showed body weight gains that were comparable to the controls. Mice: no deaths were observed in PMEA treated mice. The male and female mice (500 mg/kg) showed decreased activity and the males showed hunched bodies. After recovering within 35 min, the treated mice appeared clinically normal for the remainder of the period. All treated mice showed body weight gains comparable to control mice.

Comments: After intravenous administration of PMEA, the estimated single minimum lethal dose in male and female rats was 500 mg/kg and greater than 500 mg/kg in mice.

3. Single dose subcutaneous and intramuscular study in rabbits

with GS0393, Lot # 1965-BL-1, _____ February
24, 1993, — 450-GS-002-91. — 450A)

Groups of male rabbits (age: 8-12 weeks; strain: New Zealand White; weight: 2.2-2.7 kg; 8 animals/group) were injected with a single dose (1.0 ml) of test article via sc or im at dose levels of 25, 50 or 75 mg/kg. The animals were distributed into six groups and exposed to a regimen according to Table 1. Blood samples (1.0 ml) were collected by the marginal ear vein from two animals per group at 0.25, 0.5, 1, 2, 4, 6 and 24 hr post-dose. The purpose of the study was to evaluate the local effects of single sc and im injections of PMEA.

Table 1
Study Design of the Single Dose Study of GS0393 in Rabbits

Group #	Dose (mg/kg)	Route	No. of Rabbits/Sacrifice Period (hr)			
			24	48	72	7 days
I	25	sc	2	2	2	2
II	50	sc	2	2	2	2
III	75	sc	2	2	2	2
IV	25	im	2	2	2	2
V	50	im	2	2	2	2
VI	75	im	2	2	2	2

All animals survived until the scheduled termination of the study. In the sc-treated animals, no clinical signs were observed. Terminal necropsy revealed mottled lungs at low and high dose levels. Histopathologically at 48 hr, a slight mononuclear cell infiltration was present in one injection site and minimal to moderate hemorrhage was present in three different sites. In the im-treated animals, no clinical signs were observed in any animal during the study. Terminal necropsy revealed mottled lungs at low and mid dose levels. Minimal to slight polymorphonuclear cell infiltration, minimal to slight myofiber degeneration and/or slight edema were present in individual injection sites. At 72 hr, similar changes were still present in individual injection sites which received 50 or 75 mg/ml of PMEA. At day 7, one injection site in a low dose rabbit had a minimum polymorphonuclear cell infiltration and one high dose rabbit had an area of minimal myofiber regeneration.

Comments: Single sc or im injections (1.0 ml) of 25, 50 or 75

mg/kg PMEA in the rabbits resulted in minimal to slight local effects at 24, 48 and 72 hr. These changes were usually resolved at 72 hr; however in some cases, the changes continued to persist even until day 7. Based upon the results of the single dose sc or im PMEA administration in rabbits, the test compound is found to be a local irritant.

**4. Intravenous Single-Dose Pilot Study in the Monkey, — -41671
HPMPC, Lot # 27767-30, and — 40085 PMEA, Lot # 27962-013a,
July, 1989, —
21128/89026)**

Groups of acclimatized male and female cynomolgus monkeys (weight: 3.8 - 5.5 kg; age: unknown, 1 animal/group) were administered a single intravenous dose via a saphenous vein in a total volume of <6.5 ml/kg at a rate of 0.1 ml/sec of either HPMPC (150 or 400 mg/kg) or PMEA (150 or 500 mg/kg) in 0.9% sodium chloride. A control group was not included in this study. The objective of the study was to investigate the acute toxicity of HPMPC and PMEA following a single IV injection. With HPMPC, both single doses were lethal within 5 - 6 days after the treatment. For PMEA, at the 150 mg/kg dose, salivation and slightly labored respiration were noted during and for a few minutes after injection. Thereafter, the monkey appeared clinically normal. The animal treated with 500 mg/kg of PMEA appeared normal during and after treatment. Moderately decreased activity was apparent 2 days after treatment and this monkey was found dead the following morning. The major anatomical findings consisted of marked acute tubular necrosis primarily involving but not limited to cortical proximal tubules of the kidney, thymic lymphoid depletion and moderate hepatocellular fatty change.

Comments: The single lethal dose is, therefore, estimated to be less than 150 mg/kg for HPMPC. With PMEA, a single lethal dose is estimated to be greater than 150 mg/kg but less than 500 mg/kg.

**5. Single-Dose Intravenous Toxicity Studies in Monkeys, — -40085
(PMEA), Lot # 27962-013a, and — 41671 (HPMPC), Lot # 27411-48-
March 6, 1990, —
21499/WIL-124005)***

Groups of male and female cynomolgus monkeys (weight: 3.0 - 4.4 kg; age: adult; one animal/sex/group unless noted) were administered a single intravenous injection of either HPMPC or PMEA in a total volume of less than 6.41 ml in 1N NaOH: PMEA [75 mg (one female) and 175 mg/kg] and HPMPC [40 mg (one female) and 75 mg/kg]. Monkeys were observed for toxic effects for a period of time that ranged from 14 - 21 days. There were no control animals for either of the test compounds. The purpose of the

cecal gland (individual cell necrosis) in the low dose group animals, a NOAEL in this study could not be identified. The NOAEL should be somewhere between 0 and 5 mg/kg/day dose levels. Based on a body surface area conversion factor, an equivalent dose in humans would be between 0.0 and 0.40 mg/kg/day.

7. A 13-week oral gavage toxicity study of bis-POM PMEA in the albino mouse, Lot # 2166-A6P, February 24, 1998, (97-TOX-0840-002/T0840-00020)

Groups of male and female CD-1 mice [Crl:CD-1(ICR)BR; 15 animals/group/sex] were dosed daily via oral gavage at dose levels of 0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg/day (high) for 13 consecutive weeks. The toxicokinetic cohort consisted of 32 male mice (low and mid), and 32 male and 32 female mice (high). Blood samples were taken at predose, 0.5, 1, 2, 3, 4, 8 and 24 hr postdose on the first days of weeks 1 and 13. Concentration of bis-POM PMEA in plasma was determined using a validated method with fluorescence derivatization. Results: one male (mid) was euthanized in moribund condition on study day 8. Gross examination revealed evidence of trauma to the cranium that was considered not to be drug-related. This animal was replaced on the study. Clinical Signs: were thin fur cover, fur staining and fur un-groomed in a few control and treated animals. Body Weights: group mean body weights of the treated groups were not significantly different from the controls. The body weight gains for the females were unaffected. For the males, although not statistically significant, there was a slight, dose-related decrease (maximum 6% in the high dose group at the end of week 13) in overall body weight gain from week 0-13 for all treated groups. Food Consumption: there were no drug-related changes in food consumption seen during the treatment period. A significant decreased food consumption value noted in the males (low) from weeks 5-7 and significantly increased food consumption value noted for the females (high) from week 0-1 were considered to be incidental. Hematological Parameters: statistically significant ($p < 0.05$) drug-related changes in males and females (high) consisted of decreased red blood cell count, hemoglobin and hematocrit and increased mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, reticulocyte count and platelet count. At the mid dose, mean corpuscular hemoglobin values were significantly increased for the females and reticulocytes were increased in both males and females. Biochemical Parameters: statistically significant ($p < 0.05-0.001$) drug-related changes occurred in males (high; increased ALT, AST, AP, A/G ratio, sodium, chloride, phosphorus and creatinine and decreased glucose, globulin, cholesterol and triglycerides), females (high; increased ALT, AST, AP, A/G ratio, sodium and chloride, and decreased glucose and cholesterol), males (mid;

increased ALT, sodium and phosphorus, and decreased glucose, globulin, cholesterol and triglycerides) and females (mid; increased ALT, AST and sodium, and decreased glucose). The changes in the enzyme levels are shown in Table 2.

Table 2

Changes in enzymes for bis-POM PMEA in plasma in the 13-week oral toxicity study of bis-POM PMEA in mice (week 14)

Dose Level (mg/kg/day)	AST (U/L)		ALT (U/L)		ALP (U/L)	
	Male	Female	Male	Female	Male	Female
0 (Controls)	71.1	95	23.1	26.8	57.2	117.8
10 (Low)	73.9	86.5	27.9	32.7	57.2	101.4
30 (Mid)	89.7	497.3***	37.1**	170.6***	99.3	155.1
100 (High)	465.3***	394.6***	296.6***	270.5***	212***	256.7**

** = p<0.01; *** = p<0.001

Bone Marrow Evaluation: a drug-related increase in M:E ratio was observed in 8/12 males and 8/10 females evaluated from the high dose group when compared with the controls. Toxicokinetic Parameters: are shown in Table 3. Following the first dose, concentrations of bis-POM PMEA in plasma reached a peak at 0.5 to 1 hr post dose and declined with half-lives of approximately 3.3 to 7.7 hr, regardless of the dose level. Following repeated administration of bis-POM PMEA to mice over the dose range of 10 to 100 mg/kg/day, systemic exposure to bis-POM PMEA was dose proportional. There were no apparent differences between the pharmacokinetic parameters of bis-POM PMEA in male and female mice at the 100 mg/kg/day bis-POM PMEA dose level.

Table 3

Pharmacokinetic parameters for PMEA in plasma in the 13-week oral toxicity study of bis-POM PMEA in mice

Parameters	Dose (mg/kg/day)							
	10		30		100			
	male		male		male		female	
weeks	1	13	1	13	1	13	1	13
Cmax (µg/ml)	0.31	0.3	0.85	1.39	3.32	6.32	4.8	6.89
Tmax (hr)	0.5	1	1	0.5	0.5	0.5	0.5	0.5

Half-life (hr)	3.23	7.24	3.73	5.69	4.53	4.58	4.98	4.53
AUC _{0-∞} (µg*hr/ml)	1.55	1.61	4.33	7.37	16.31	24.1	15.5	25.17
AUC/Dose	0.155		0.144		0.163		0.155	
F (%) using historical AUC _{0-∞} values	23.29		21.75		24.59		23.37	

Organ Weight Assessment: liver weights (both absolute and body weight relative) were significantly increased ($p < 0.05$) and thymus weights (both absolute and body weight relative) were significantly decreased in the high dose animals. Spleen weights (both absolute and body weight relative) were significantly increased in the high dose males, whereas only body weight relative spleen weights were significantly increased in the high dose females. Body weight, relative kidney and adrenal weights were significantly increased for the high dose males. At the mid dose, absolute thymus weights for the males were significantly reduced. Gross Pathology: changes were observed at a higher incidence or exclusively in the high dose animals and affected primarily the males. These findings were seen in the liver (discoloration, pale-9/15 males, 1/15 females), kidney (discoloration pale-4/15 males; enlargement-4/15 males), thymus (small-3/15 males, 2/15 females) and spleen (enlarged-2/15 males, 3/15 females). Histopathological Changes: considered to be drug-related were observed at all dose levels. In the liver, hepatocellular changes described as karyo/cytomegaly were observed in 15/15 males and females (high; severity-slight to severe), 12/15 males and 11/15 females (mid; severity-slight to moderate) and 3/15 males and 2/15 females (low; severity-slight). In addition, single cell necrosis was observed in 14/15 males and females (high; severity-slight to moderate), 3/15 males and 8/15 females (mid; severity-slight to mild) and 2/15 males and females (low; severity-slight). Also, oval cell hyperplasia was observed in 14/15 males and 11/15 females (high; severity-slight to moderate) and 1/15 males and 7/15 females (mid; severity-slight). Two females (high) had a few hyperplastic hepatocellular foci. In the kidney, karyo/cytomegaly of the cortical tubular cells was seen in 15/15 males and females (high; severity-slight to moderate) and 5/15 males and 2/15 females (mid; severity-slight to mild). Degeneration/regeneration changes involving the tubules were observed in 15/15 males and 9/15 females (high) and 2/15 males (mid). Widespread, slight to moderate dilatation of cortical tubules was evident in 14/15 males and 2/15 females (high). In the bone marrow, a slight change described as erythroid hypocellularity was observed in 9/15 males and 5/15 females (high) and 2/15 females (mid). Thymic lymphoid necrosis/atrophy was seen in the thymus of 9/15 males and 11/15 females (high). Increased hematopoiesis was seen in 4/15 males

and 1/15 females (high).

Comments: Based on the histopathological changes present in the liver (hepatocellular karyo/cytomegaly, single cell necrosis) and spleen (lymphoid necrosis/atrophy) in the low dose group animals, a NOAEL in this study could not be identified. The NOAEL should be somewhere between 0 and 10 mg/kg/day dose levels. In addition, based on the incidence and severity of histopathological changes present in the liver in the low dose group, a MTD for a two-year oral carcinogenicity study of bis-POM PMEA in mice would appear to be 10 mg/kg/day. The major target organs of toxicity associated with bis-POM PMEA treatment were liver, kidney, spleen/thymus and bone marrow.

8. 14-day oral toxicity study of bis-POMPMEA in rats, Lot # GS-840-01, February 11, 1994, — 437-GS-001-93/T0840-00002)

Groups of male and female Sprague Dawley rats (weights: 107-174 g; 5 rats/sex/group) were administered bis-POMPMEA via oral gavage in either PEG-based formulations at dose levels of 0 (vehicle control), 11 (low), 37 (mid) or 110 mg/kg/day (high) or in an aqueous suspension formulation (without propylene glycol) at a dose level of 110 mg/kg/day (high, suspension) for 14 consecutive days. Results: in the high-dose suspension group, one male and one female were sacrificed in extremis on day 9. Clinical signs of toxicity consisted of piloerection and poor grooming, post dose salivation, abnormal stance, abnormal gait, flaccid body tone, chromodacryorrhea and dehydration (mid and high, high suspension). The incidence and severity of clinical signs were dose dependent with the greatest signs of systemic toxicity observed in the high dose suspension group. Other clinical signs of toxicity consisted of a dose-related decrease in group body weights, body weight gains and food consumption in the mid-, high- and high-dose suspension groups. Kidney to body weight ratios were also increased in male and female rats in the high- and high-dose suspension groups. Food consumption was also decreased in the low- and mid-dose females. Treatment-related clinical pathology changes in males included decreased total protein, calcium, white blood cells, mean corpuscular volume and lymphocytes (high, high suspension). In female rats, clinical pathology changes consisted of decreased mean corpuscular volume and increased blood urea nitrogen and ALP (high) and increased chloride and bilirubin (mid, high and high suspension). Histopathology: treatment-related lesions were present in the kidneys and gastrointestinal tracts of the male and female rats (mid, high and high suspension). The treatment-related changes in the kidneys (mid, high and high suspension) were of similar incidence and severity. These changes were tubular depletion/degeneration, tubular cytomegaly, tubular karyomegaly

and an increased incidence and severity of tubular regeneration and tubular dilatation. Treatment-related changes were present in stomachs of male and female rats (mid, high and high suspension). In the high dose groups, there were similar incidences and severity of degeneration of the glandular mucosa accompanied by regeneration/hyperplasia. These changes were more pronounced in the pyloric portion of the stomach than in the fundic portion. There were ulceration and hyperplasia of the non-glandular stomach in three rats and hyperplasia of the non-glandular stomach in a fourth rat (high, suspension). In the mid dose group, the changes were limited to degeneration of the glandular mucosa, primarily in the pyloric portion of the stomach. In small intestines of male and female rats (mid, high, high suspension), there were changes in the duodenum characterized by erosions and glandular regeneration/hyperplasia of the glandular mucosa and villous fusion. In the two high dose groups, there were villous fusion and/or villous atrophy in the jejunum and ileum, and villous fusion and goblet cell hyperplasia in the cecum and colon. The cause of morbidity of a male and a female rat (high suspension) was considered to be related to the stomach lesions.

Comments: An oral dose of 11 mg/kg/day may be considered a NOEL. Based on the body surface area, an equivalent dose for a 60 kg person would be 1.6 mg/kg/day. Kidney and gastrointestinal tract were identified as the target organs.

9. A 14-day oral gavage toxicity study of bis-POMPMEA in the albino rat, Lot # TX840-97-05, April 8, 1998, (97-TOX-0840-006)

Groups of male Sprague Dawley rats (strain: Cr1: CD(SD)BR; weights: 183-201 g; 5 rats/group) were administered bis-POMPMEA via oral gavage at dose levels of 0 (vehicle control), 12 (low) or 37 mg/kg/day (high) for 14 consecutive days. Results: there were no deaths in the study. There were no treatment-related clinical signs of toxicity nor was there any effect on food consumption, hematology and clinical chemistry parameters. Group mean body weight gains were statistically lower (high) when compared to the controls. Absolute and relative kidney weights were statistically higher (high) than the controls. Histopathology: kidneys (high) were pale and exhibited slight to moderate lesions (5/5) characterized by tubular cell karyomegaly, tubular dilatation and tubular degeneration/regeneration. All animals (low) were observed to have slight tubular cell karyomegaly, but one animal (low) exhibited a slight tubular degeneration/regeneration.

Comments: A NOAEL could not be identified in this study. Renal lesions were less severe in the 12 mg/kg/day dose level. Based on a body surface area conversion factor, an equivalent dose for a

60 kg person would be 1.94 mg/kg/day.

10. A 4-Week Oral Toxicity Study with bis-POMPMEA in Rats, Lot # JM-614-5, January 6, 1994, (6511-101/93-TOX-0840-001)

Groups of male and female rats [weight: 141-255 g; age: 6 weeks; strain: Sprague-Dawley Crl:CD(SD)BR] were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control, 8 males and 5 females), 4 (low, 12 males and 5 females), 12 (mid, 12 males and 5 females) or 37 mg/kg (high, 12 males and 5 females) for a period of 28 days. Results: all animals survived to the terminal sacrifice on day 30. No drug-related clinical signs of toxicity occurred in any dose group. The treatment resulted in statistically significant lower body weights (7.5%; $p=0.05$), decreased cumulative body weight gains and food consumptions ($p=0.05$) for males and females (high). Clinical pathology: findings consisted of lower urine pH (♀) and glucosuria (♂; high). Pathology: there was an increased incidence of macroscopic renal findings. The kidneys were diffusely light-colored, large and contained cysts (high). Drug-related histomorphologic changes were noted in the kidneys of males (mid or high) and females (high). The tubular nephrosis (5/5♂; 5/5♀; high) was characterized by megalocytosis, tubular degeneration, tubular dilatation, or lymphohistiocytic infiltrates. The changes (high) in males were generally more severe than in females. In males (5/5♂; mid), the most prominent feature was megalocytosis. Toxicokinetics: demonstrated an AUC (♂) of 6.32 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at the high dose of 37 mg/kg/day. There were insufficient data and/or assay sensitivity to derive AUC values at the lower doses studied (4 and 12 mg/kg/day)

Comments: As evident from the macroscopic and microscopic examinations, the kidney is the target organ. A dose level of 4.0 mg/kg/day may be considered the NOEL for this study. With a dose conversion based on body surface area, the equivalent oral dose for humans would be 0.6 mg/kg/day.

11. A 6-Month chronic oral toxicity study of bis-POMPMEA in Rats, Lot # 808-GS-93, May 2, 1996, (94-TOX-0840-003\Project No. 86539)

Groups of male and female rats [strain: Sprague-Dawley Crl:CD(SD)BR; age: 6 weeks] were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control), 0.4 (low), 2.0 (mid) or 10.0 mg/kg/day (high) for 13 or 26 consecutive weeks. Fifteen rats/sex/group were assigned to the main study (26-week) treatment cohort and 10 rats/sex/group were assigned to the interim necropsy (13-week) treatment cohort. Five rats/sex/group were assigned to each control and high dose groups

for each post-dose (4-week) non-treatment recovery period. An additional cohort of 14 male rats each in the low, mid and high and 14 female rats (high) were designated for toxicokinetic analysis only. Toxicokinetic blood samples (2 rats/time point) were taken pre-dose and at 1, 2, 4, 6 and 12 hr post-dose on study day 1 and during weeks 12 and 26. Results: eight animals were found dead or euthanized moribund prior to scheduled termination: 1 male (low), 3 males (mid) 1 male (high) and 3 females (high). Three deaths were attributed to the blood collection procedure. Two males (mid) and 3 females (high) were found dead (no treatment-related histopathological findings) in week 10 or 13 of the treatment period. One male (high) was found dead (histopathological findings: tubular karyomegaly) during week 26. Clinical signs: incidental findings found in a few control and/or treated animals included thin fur cover, fur staining and skin scabs on the limbs, paw or muzzle. A mass was seen in the urogenital region of 1 male rat (control) during week 26 which correlated with the preputial abscess seen histopathologically. A soft mass located in the left urogenital region of one male rat (high) was noted during week 7; this mass was not observed at necropsy and therefore could not be correlated to any gross or histopathological findings. A firm red mass on the muzzle of one male rat (high, toxicokinetic analysis) was noted during week 17; this animal was euthanized without a gross necropsy observation (no histopathological evaluation). Body weights: there were no treatment-related changes in the group mean body weights of treated animals when compared to the respective control groups. Food consumption: relative to control group values, statistically significant decreases ($p=0.05$) in food consumption were seen in males treated (high) for weeks 1, 23 and in females (mid) for week 7. Laboratory investigations: no treatment-related changes. Organ weights: an increase in absolute kidney weights (high) was observed during week 27 which corresponded to the slight-to-mild tubular karyomegaly seen histopathologically. An increase in absolute spleen weights was observed in females (low and mid) and kidney weights (high). A decrease in pituitary weights (relative to body weight) and an increase in spleen weights (relative to brain weight) were seen in females (mid). Gross findings: the most frequent change was the presence of pale area(s) in the liver, for which there was no histopathological correlation. Microscopic findings: revealed only a slight-to-mild renal tubular karyomegaly in the high dose male and female rats of the interim, main and both recovery groups. Incidence of renal tubular karyomegaly in the high dose group: interim study-10/10 ♂ and 8/10 ♀, interim recovery-5/5 ♂ and 1/5 ♀; main study-15/15 ♂ and 14/15 ♀, and main recovery-5/5 ♂ and 3/5 ♀. Tubular karyomegaly was characterized histologically by tubular cells with an enlarged nucleus and often prominent nucleolus and was seen mainly in tubules of the outer cortex. Toxicokinetics: AUC values are shown in Table 4.

Table 4

AUC values for PMEA in plasma following repeated daily oral administrations of bis-POM PMEA to male rats

Dose (mg/kg/day)	Group	AUC _{0-∞} (µg*hr/ml)		
		Week 1	Week 12	Week 26
0.4	Low	nd	nd	nd
2.0	Mid	1.09	1.4	1.42
10.0	High	1.14	1.81	1.82

nd: not detected

Comments: Based on results from the present study, the NOEL for bis-POMPMEA in rats following oral gavage administration once daily for up to 26 consecutive weeks was 2 mg/kg/day. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 0.33 mg/kg/day. Kidney was identified as the target organ in both male and female rats.

12. A 4-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-5, January 5, 1994, (6511-100/93-TOX-0840-002)

Groups of male and female cynomolgus monkeys (weight: 2.0-3.8 kg; age: adult; 4 animals/sex/group) were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control), 8 (low), 25 (mid) or 75 mg/kg (high) for a period of 28 days. All animals survived to the terminal sacrifice on day 30. Drug-related observations included changes in fecal consistency (non-formed, liquid or mucoid feces) and vomitus (all groups). Body weights were significantly lower for males (high) on days 11-25, females (mid) on day 18, and females (high) on day 25. A dose-related decrease in food consumption was noted. In male and female animals, dose-related increases in AST and ALT values (<3.5-fold, high) and CK values (3- and 11-fold males and females, respectively, high) were observed. Macroscopic Observations: an increased incidence of changes in the kidney for females (high) was observed. Three of the four animals (high) exhibited one or more of the following kidney changes: diffusely light-colored, large, raised area(s) and adhesions. Microscopic Observations: drug-related changes were seen in the kidney and stomach. In the kidney, a tubular nephrosis, characterized by megalocytosis or vacuolization or tubular cell enlargement was noted in the majority of males and females (mid and high). A minimal change, consisting of megalocytosis only, was detected in one male (low). In the stomach, hyperplastic, degenerative and inflammatory changes were seen in all treated groups. The

epithelial hyperplasia consisted of increased cytoplasmic basophilia, increased mitoses, anisokaryosis and decreased apparent numbers of parietal cells. Minimal to slight degeneration/necrosis of gastric epithelium was also found, affecting individual cells. The inflammatory changes was characterized by increased infiltration of lymphohistiocytic cells, often with prominent areas of neutrophil infiltration.

Comments: A NOEL could not be identified in this study. In monkey, the primary target organs were kidney and stomach.

13. A 13-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-70-11, September 1, 1994, (/6511-103/94-TOX-0840-001)

Groups of male and female cynomolgus monkeys (weight: 2.0-3.7 kg) were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control, 6 animals/sex), 1 (low, 4 animals/sex), 5 (mid, 4 animals/sex) or 25 mg/kg (high, 8 animals/sex) for a period of 13 weeks. Two animals/sex (high) were placed on recovery for 4 weeks following the first 4 weeks of treatment; and two animals/sex (control and high) were placed on recovery for 4 weeks following 13 weeks of treatment. For drug absorption determination, blood was collected at 0, 0.5, 1, 2, 4, 6 and 12 hr post dose on days 1, 25, 53 and 84. Results: all animals survived to their respective scheduled sacrifice date. Clinical signs: drug-related observations included changes in fecal consistency (non-formed, liquid or mucoid feces) and vomitus (all groups). Food consumption: there was a lower incidence of food consumption (not dose-related) in all drug-treated female animals. Clinical pathology: males and females (high) had higher (2 to 3-fold) serum AST and ALT values, and CK values (2 to 9-fold). The higher serum CK activity was predominately due to increased activity of the skeletal isoenzyme (CK-MM). All enzyme effects were dose-dependent, did not noticeably change in magnitude with longer duration of drug exposure, and were reversible. Organ weights: absolute and relative kidney weights were higher for male and female animals (high). Microscopic Observations: minimal to slight nephropathy, characterized by large variable-sized nuclei and occasional individual tubular cell necrosis, was observed at the 13-week sacrifice in 3/4 males and 2/4 females (mid and high each). The lesion was most frequent and severe in high dose animals. The lesion was also seen in recovery animals: 4-week of treatment with 4-week of recovery (1 high dose female), and 13-week of treatment with 4-week of recovery (two high dose males and one female).

Comments: A dose of 1.0 mg/kg/day may be considered a NOEL for

bis-POMPMEA in this study. In monkey, the primary target organ is kidney. Liver enzyme activity was increased in high dose animals; and it was reversible in four weeks of recovery period. Probenecid in the high dose group may be partly responsible for increased serum CK activity. Probenecid decreases serum uric acid levels, which in turn can lead to increased CK levels since uric acid is an inhibitor of CK activity.

14. A 52-Week Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # TX840-96-01, February 5, 1998, (T0840-00019/95-TOX-0840-006)

Groups of male and female cynomolgus monkeys (weight: 2.0-2.9 kg; 5 animals/sex/group) were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control), 0.2 (low), 1.0 (mid) or 5 mg/kg/day (high) for a period of 52 weeks. A recovery cohort (2 animals/sex/group) in low and high dosage groups were retained for 4-non-dosing weeks following the 52 treatment weeks. For drug absorption determination, blood was collected at 0, 0.5, 1, 2, 4, 6, 12 and 24 hr post dosing on day 1 and during weeks 13, 26, 39 and 52. Results: all animals survived to their respective scheduled sacrifice date. Clinical signs: incidental clinical signs seen in the controls or treated groups included emesis, skin redness, scabs or lesions and/or thin fur cover of the hindlimb or forelimbs. Liquid feces were seen moderately frequently in both the controls and treated animals. Body weights and food consumption: treatment-related changes in the group mean body weights and in appetency were not seen during the treatment or recovery phases when compared to the controls. Hematology: red blood cell counts, hemoglobin and hematocrit values were reduced (p=0.5) in the treated groups compared to the controls from study weeks 13 through 52. However, these erythroid cell changes, which were not dose-related and did not increase in severity with treatment duration, are believed to reflect a transient anemia caused by pharmacokinetic blood sampling (approximately 8 ml/animal/sample day) 2-to-3 days prior to clinical pathology blood sampling. There was no pharmacokinetic blood sampling of animals administered the control article formulation. During week 13, statistically significant increases were seen in the prothrombin time of males (high). During week 52, statistically significant decreases were seen in the red cell distribution width of males (high). During week 26, statistically significant increases were seen in the mean corpuscular hemoglobin levels of males (mid). Statistically significant decreases were seen in the activated partial thromboplastin times of males and females (low, mid or high) during weeks 26 and 52. Bone marrow smears: treatment-related changes (low, mid or high) were a slight decrease in the number of mature erythroid cells (primarily the orthochromatic erythroblasts) and a very slight increase in the

myeloid:erythroid ratio when compared to the controls. These changes could suggest a slight decrease in erythropoiesis. Clinical biochemistry: statistically significant changes associated with the test article administration consisted of 2- to-3 fold increases in ALT (high) from weeks 13 through 52. Creatine kinase (CK) activity in week 52 samples showed a statistically significant increase (17-fold) in males (high). ALT and CK values in the high dose males recovery cohorts returned to normals levels; the ALT values in females remained elevated. Statistically significant increases in other clinical chemistry parameters (high) were reported at single timepoints and not at the end of the treatment (ie, AST in males in study week 26 and in females in study week 39; chloride in males and females in study week 39). There was an apparent decrease in cholesterol in females (mid) at weeks 39 and 52. Organ weights: there were no treatment-related changes seen in the organ weights. Gross and microscopic observations: there were no gross pathological findings seen which were related to the test article. Histopathological changes associated with the test article were present only in the kidneys (high). A slightly severe cortical tubular cell karyomegaly, characterized by a slight increase in nuclear size of epithelial cells with slight anisokaryosis, was present in 3 of 5 males and 4 of 5 females (high). This renal lesion was present in 2 of 2 recovery females (high). No evidence of hepatocellular lipid accumulation was observed in liver tissue sections stained with Oil Red O.

Comments: A dose of 1.0 mg/kg/day may be considered a NOAEL for bis-POMPMEA in this study. Based on a body surface area factor, an equivalent dose in humans would be 0.33 mg/kg/day. In monkey, the primary target organs were bone marrow and kidney. Liver enzyme activity was increased in high dose animals; it was not completely reversible in the four weeks of recovery period. In the clinic, the proposed dosages of the test compound are 60 and 120 mg/day.

15. Serum Carnitine Levels in Monkeys Administered Adefovir dipivoxil for 13 and 52 Weeks in Toxicity Studies (TOX-99-0840-001)

The effects of adefovir dipivoxil administration on carnitine levels in cynomolgus monkeys were evaluated in separate 13-week (94-TOX-001) and 52-week (95-TOX-006) oral toxicity studies. Total and free carnitine levels were measured at termination of dosing and at the end of a 4-week recovery period. Results: of the analysis are shown in Table 5 and 6. Dose- and time-related reduction in total and free carnitine levels were observed after 13 or 52 weeks of adefovir dipivoxil administration to cynomolgus monkeys. Following a 4-week drug-free recovery period in both studies, total and free carnitine levels in the high dose monkeys

showed partial reversal in carnitine deficits relative to the controls. In either study, there was no histological evidence of changes in the liver or muscle that might be considered secondary to reductions in carnitine levels. Liver sections (high) from the two studies showed no evidence of hepatocellular lipid accumulation. In addition, there were no clinical symptoms (eg, lethargy or inactivity) in the monkeys potentially related to low levels of carnitine levels. Conclusions: treatment with adefovir dipivoxil at > 1 mg/kg/day for 13 or 52 weeks in cynomolgus monkeys caused decreased total and free carnitine levels; partial resolution of the carnitine deficits occurred in the treated animals relative to the controls after 4-weeks of recovery period.

Table 5

Serum carnitine levels in monkeys administered adefovir dipivoxil for 13 weeks, following a 4-week recovery period

Dose level (mg/kg/day) & sex	Total carnitine (nmole/ml) week 14		Total carnitine (nmole/ml) week 18		Free carnitine (nmole/ml) week 14		Free carnitine (nmole/ml) week 18	
	Mean	% control	Mean	% control	Mean	% control	Mean	% control
0, ♂	46.4	100	34.4	100	44.7	100	33.4	100
0, ♀	40.7	100	54.2	100	36.9	100	47.4	100
1, ♂	48.1	104	-	-	43.4	97	-	-
1, ♀	33.9	83	-	-	29.4	80	-	-
5, ♂	26.8	58	-	-	24.2	54	-	-
5, ♀	21.4	53	-	-	19.6	53	-	-
25, ♂	8.9	19	9.4	27	7.7	17	8.4	25
25, ♀	7.2	18	11.4	21	6.1	17	10.6	22

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Table 6

Serum carnitine levels in monkeys administered adefovir dipivoxil for 52 weeks, following a 4-week recovery period

Dose level (mg/kg/day) & sex	Total carnitine (nmole/ml) week 52		Total carnitine (nmole/ml) week 56		Free carnitine (nmole/ml) week 52		Free carnitine (nmole/ml) week 56	
	Mean	% control	Mean	% control	Mean	% control	Mean	% control
0, ♂	40.5	100	36.8	100	30.2	100	31.9	100
0, ♀	37.3	100	30.1	100	27.4	100	24.5	100
0.2, ♂	38.2	94	-	-	31.2	1.3	-	-
0.2, ♀	37.3	100	-	-	28.3	1.3	-	-
1, ♂	30.3	75	-	-	22	73	-	-
1, ♀	34.9	94	-	-	25	91	-	-
5, ♂	11.8	29	15.8	43	10.1	33	14.8	46
5, ♀	9.8	26	25.4	84	7.4	27	20.6	84

16. Intravenous Multiple-Dose (x5) Range Study in Rats, — 41671 (HPMPC), Batch # 26870-064 and — -40085 (PMEA) Phosphonates, Batch # 26870-059, October 3, 1988, — -20887/88032)

Groups of male Sprague-Dawley (CrI:CD (SD) BR) rats (weight: 136 - 160 g; age: 6 - 7 weeks, 5 animals/group) were administered intravenously via the tail vein in a total volume of 0.25 ml/100 g at a rate of 0.05 ml/sec either HPMPC or PMEA in 0.9% sodium chloride at a daily doses of either 10, 100 or 250 mg/kg/day for 5 consecutive days. Following treatment, the rats were observed for 10 days. A control group (5 animals) received 0.9% sodium chloride for injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA in the male rats. As result of HPMPC toxicity, 3 of the 5 rats died 4 - 6 days after treatment (high dose). Body weight losses or significantly decreased weight gains were noted in the intermediate and high dose groups. Dose-related nephrotoxicity (cortical tubular nephrosis) was apparent in the intermediate and high dose groups. Other major findings included a transient leukocyte reduction due to a decrease in lymphocytes and slight lymphoid depletion at the high dose group, and a slight increase in mitotic figures in liver sections at the intermediate dose level. At the end of the observation period, elevations in ALT were evident in the intermediate and high dose groups. In the low-dose animals, a slight increases in mitotic figures in liver sections in one rat were detected. On the other hand in PMEA treated animals, transient and dose dependent

decreases in body weight gain occurred during treatment in the intermediate and high dose groups. Nephrotoxicity was detected at the high dose level and correlated with increased BUN. Other toxicity included slight transient leukocyte reduction, decrease in neutrophils in both the intermediate and high dose groups, slight lymphoid depletion, reticulocytopenia, slight elevations in ALT and increased mitotic figures in liver sections in the high dose group. No toxicity was observed at the low dose.

At necropsy, pale kidney cortices were noted for 4 intermediate and 2 high dose HPMPC as well as 2 high dose PMEA-treated rats; evidence of gastro-intestinal hemorrhage or ulceration and/or liquid/watery intestinal contents were noted for 3 high dose HPMPC and 2 high dose PMEA treated rats. Histopathological findings included a dosage related tubular nephrosis in kidney sections from the intermediate and high dose HPMPC and the high dose PMEA-treated rats, lymphoid depletion in thymic and/or splenic sections from 3 high dose HPMPC and 3 high dose PMEA-treated rats, and increased mitotic figures in liver sections from one or more low and intermediate dose HPMPC and one of the high dose PMEA-treated rats.

Comments: The death of high dose HPMPC-treated animals may be attributed to tubular nephrosis. Nephrotoxicity appears to be major dose limiting toxicity. A dose of 10 mg/kg/day of PMEA or HPMPC may be considered a NOAEL; an equivalent dose in humans would be 1.4 mg/kg/day. For PMEA (high dose) animals, the observed nephrotoxicity (cortical tubular nephrosis) was correlated with increased BUN.

17. Fourteen day repeated dose oral toxicity study of PMPA and PMEA in Sprague-Dawley rats, Lot # 891-161-32, Gilead Science, Inc., Foster City, CA, November 30, 1995, (95-TOX-1278-001)*

Groups of male Sprague-Dawley rats (5/group) were orally gavaged with PMPA at dose levels of 0 (control vehicle), 10 (low), 50 (mid) or 250 mg/kg/day (high), or PMEA at dose levels of 50 (low) and 250 mg/kg/day (high) once daily for 14 days. Results: two unscheduled deaths occurred on study: one rat (low, PMPA) died (gavage accident) immediately after dosing on study day 8 and one animal (low, PMPA) sacrificed *in extremis* on study day 11. This animal had histopathologic changes (severe chronic active pleuritis and pericarditis) consistent with a gavage accident. No test article-related clinical signs of toxicity were present in any PMPA or PMEA treatment group. No test article-related changes in clinical chemistry and hematology values were noticed in any dose group. Histopathology: changes consisted of mild to severe necrosis of proximal convoluted tubules of the kidneys (high, PMEA).

Comments: Based on results from this study, the NOELs for oral PMPA and PMEA were 250 mg/kg/day and 50 mg/kg/day, respectively. Based on a body surface conversion factor, an equivalent dose of oral PMPA and PMEA in humans would be 35.7 mg/kg/day and 7.1 mg/kg/day, respectively.

18. Multiple-Dose Intravenous Pilot Study in Rats, —-41671 (HPMPC), Lot # 26870-64 and —40085 (PMEA), Lot # 26870-059, January, 1989, (——— 20971/88072)

Groups of male Sprague-Dawley (Cr1:CD (SD) BR) rats (weight: 175 - 220 g; age: 43 days, 5 animals/group) were administered drug intravenously for 14 consecutive days via the tail vein in a total volume of 1.0 ml/kg at a rate of 0.05 ml/sec either HPMPC (0.3, 1.0, 3.0, 10.0, or 50 mg/kg/day) or PMEA (1.0, 10.0 or 50 mg/kg/day) in 0.9% sodium chloride. Following treatment, rats were observed for 10 days. Control groups received 0.9% sodium chloride for the injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA treatment for 14 days and to select appropriate dose levels for a one-month toxicity study in this species. With HPMPC, doses of 10 or 50 mg/kg/day resulted in a significant depression of body weight gain. Overt toxicity consisting of hunched body posture, dehydration and emaciation occurred during the 2nd week of the drug treatment at 50 mg/kg/day. Nephrotoxicity characterized by tubular nephrosis was observed at doses of 3 mg/kg/day or more and severity was dose-dependent. Elevations in BUN, creatinine and AST and a decrease in bone marrow cellularity were apparent at 10 and 50 mg/kg/day. Additional findings noted at 50 mg/kg/day consisted of decreases in hematocrit, total leukocyte count, percent and absolute lymphocyte and reticulocyte values and lymphoid depletion. No evidence of toxicity was detected at 0.3 or 1.0 mg/kg/day. With PMEA: Significant depression of body weight gain was observed in all animals (50 mg/kg/day). Nephrotoxicity (renal tubular nephrosis), elevations in serum urea nitrogen and creatinine, decreased bone marrow cellularity and lymphoid depletion were detected at 10 and 50 mg/kg/day and severity was dose-dependent.

Comments: For both the compounds, a dose of 1 mg/kg/day for 10 days may be considered a NOAEL. With a body conversion based on body surface for humans, an equivalent dose will be 0.14 mg/kg/day.

19. One-Month Intravenous Toxicity Study in Rats, —40085 (PMEA), Identification # 27337-11 and 27337-15, January 24, 1990, (——— 21489/ — 124004)*

Four groups of normal male and female rats (CrI:CD BR) (weight: 227 - 279 g males and 159 - 195 g females; age: eight weeks; 10 animals/sex/group) were administered PMEA by intravenous injection (via lateral tail vein) 1.0, 5.0 and 15 mg/kg/day in sterile saline (0.2 ml/100 g) for a period of 29 or 30 consecutive days. Control group received sterile saline under similar experimental conditions. This study was designed to investigate the toxicological potential of PMEA when administered intravenously (once-a-day) to rats for a period of one month.

Clinical Observations, Survival and Ophthalmoscopic Examination: All animals survived to the scheduled sacrifice. One male (high dose) had red material around the nose at the time of dosing once during the second week of dosing. One female (high dose) had one occurrence of ataxia at the time of dosing during the second week; this animal also had a purulent dermal lesion on the distal end of the tail on one day during the last week of dosing. Clinical findings were also noted in two (mid dose) males; one animal had soft stool on one day prior to dosing (study week 4) and the other male was dehydrated and had decreased defecation on one day (study week 1). No oculo-pathic lesions indicative of a toxic effect were observed at any dose.

Body Weight and Food Consumption: Mean body weight gain in (high dose) animals was lower than the control group during week 0 - 1 ($p < 0.01$ females only). Body weight gains in the high dose group animals remained significantly lower than the control group during study weeks 1 - 2 and 2 - 3 ($p < 0.01$) and the weight gains continued to be significantly lower in this group of animals during rest of the study. No adverse effect on mean body weight, body weight gain or final body weight was apparent in males and females at low and mid dose groups. On occasions, mean food consumption values (g/animal/day) at all dose levels were significantly below the concurrent control mean. These differences were not large and always similar to the pretreatment means.

Hematology, Serum Chemistry and Urinalysis: No treatment-related differences in hematology parameters were apparent at any dose level. Mean glucose levels in animals (high dose) were slightly lower than the control animals (statistically not significant). Mean ALT values for males (mid and high doses) were significantly lower ($p < 0.05$) than the control values. The cholesterol mean in males (high dose) was higher ($p < 0.05$) than the control values. No treatment-related effects in urinalysis parameters were apparent.

Macroscopic and Microscopic Examination: In the high dose group males, one animal had dilated renal pelvis, one had small testes and epididymis, and one had a tissue mass in the bladder. Slight nephropathy was observed in 4 of 10 males (high dose). Nephropathy (involving the proximal convoluted tubules) was characterized by minimal hypertrophy of tubules which were lined by hyperplastic epithelia and a minimal increase in the basement membrane thickness. Occasional protein casts were present in collecting tubules at or below the corticomedullary junction. Two

males (high dose) were found to have a 5 x 2 x 2 mm tissue mass involving the urinary bladder. Organ Weight: The absolute weight and relative [to final body weight] ratios for thymus in high dose animals were decreased when compared to control animals ($p < 0.01$). A decreased absolute kidney weight was also observed in high dose males ($p < 0.05$). The brain weight relative to final body weight ratio was significantly higher ($p < 0.05$) in the high dose animals than the controls.

Comments: As evident from the macroscopic and microscopic examinations, the kidney is the major organ affected by treatment with the drug. A dose level of 5.0 mg/kg/day may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.71 mg/kg/day. The decrease in thymus weight suggests that thymus may be a possible site of toxicity in human. The increased brain weight relative to final body weight ratio suggest that body weight gain has been restricted by the treatment. The increases in ALT and cholesterol levels suggest that liver may be a possible site of toxicity for the compound.

20. Thirty day subcutaneous toxicity study of GS-0393 in rats, Lot # 1965-BL-1, March 3, 1993, (UIC/TRL-109)*

Three groups of normal male and female rats [strain: (VAF) CD; weight: 227 - 279 g males and 159 - 195 g females; age: eight weeks; 10 animals/sex/group] were administered PME A by a subcutaneous injection at 3, 20 or 40 mg/kg/day in sterile water for a period of 30 consecutive days. A control group received sterile water under similar experimental conditions. This study was designed to investigate the toxicological potential of PME A when administered sc to rats in a one month sub-chronic toxicity study. Clinical Observations and Survival: all animals survived to the scheduled sacrifice. Clinical signs of toxicity were seen only during the last week of the study and were limited to rough coat (5 males high dose and 1 male mid dose) and hunched posture (2 males high dose). Body Weights: statistically significant reductions in body weight gains were seen in all animals in mid and high dose groups. Body weight were not affected at the low dose level. Food Consumption: statistically significant reductions in food intake were noted for males and females (high) and to a lesser extent for mid dose animals. Food consumption for low dose animals was comparable to the controls. Clinical Pathology-Hematology, Serum Chemistry and Urinalysis: significant anemia, as indicated by decreased RBCs, hemoglobin, hematocrit and increased MCV, was seen in high dose and to a lesser extent mid dose animals. Reticulocyte counts (high) were decreased. A significant increase in the mean platelet counts was seen for all animals (high) and males (mid) dose groups. Dose-related