the stomach (females, very high) were observed at the week 43 sacrifice. In addition, dark areas in the mandibular lymph nodes were noted in a large number of animals from all treated groups. No gross findings were present at the end of the recovery period (week 56). Histological findings: related to the treatment were seen in the kidneys and gastrointestinal tract. Slight to mild renal tubular karyomegaly characterized by a minimal variation in size of some nuclei without any additional change in tubular epithelium was noted in all animals (very high, weeks 14 and 43). This was also seen in all animals (mid at week 14) and in some animals (low, mid and high, week 43). At week 56, this finding was still present in all males (high and very high) and in all females (very high). A dose-related increase in the incidence and severity (slight to moderate) of renal tubular pigment accumulation was noted at week 43 in animals of all dose groups including controls (1/10). Slight renal tubular pigment was observed in 1/5 males (mid) and 1/4 males (high) and 1/5 females (very high) at week 56. At week 14 sacrifice, a slight to mild epithelial hypertrophy in the duodenal mucosa was present in some animals of both sexes (very high) and featured enlarged epithelial cells with diminished spaces between individual villi, a change which was occasionally described grossly as a thickening of the duodenal wall. Mild to moderate gastritis, infrequently ulcerative, was noted in a few animals of both sexes (very high). Slight to mild typhlitis, characterized by slight inflammation with mucosal atrophy, appeared in males (high and very high). At week 43 sacrifice, the gastrointestinal changes included slight to moderate gastritis in a few animals (very high); slight to moderate typhlitis (high, males), inflammation and/or mucosal atrophy of cecum and colon (high males, very high females), mild inflammation and slight to mild epithelial hypertrophy of the jejunum in some males (very high); mild inflammation and slight villous atrophy of the ileum of a few males (very high); and slight to moderated mucosal hypoplasia (1/10 females, high; 4/10 females and 4/11 males, very high). Changes observed at weeks 14 and 43 in the gastrointestinal tract virtually disappeared by week 56 except for a mild case of submucosal typhlitis with mesenteric granulomas in one male (high). Bone evaluation: peripheral quantitative computed tomography (pQCT) revealed decreases in bone mineral contents and bone mineral density of the total slice and trabecular and cortical/subcortical areas for males and females (very high) for 13 or 42 weeks. Decreases in cortical thickness generally correlated with slight decreases in periosteal circumference and slight increases in endosteal circumference. Similar effects on the cortex were observed at the mid-femur diaphysis with evidence of a similar dose-related effect for males (high) at week 43. There was some evidence of a reversal of these changes observed for animals (high and very high) at the week 13 recovery period (ie, week 56), most notably at the femoral metaphysis region. There were no meaningful

effects at the low or mid dose on bone parameters derived by pQCT. Biochemical markers of bone turnover and bone densitometry: deoxypyridinoline was increased at various sampling time in both males and females (low or higher); deoxypyridinoline mean values were comparable to the controls during the recovery period. Osteocalcin was increased at variable sampling times in males and females (mid or higher); increases in osteocalcin were sustained during the recovery period in males (very high). Urinary calcium or phosphorus levels (normalized for creatinine) were increases at every sampling time in males and females (very high). Serum parathyroid hormone values were marginally increased (mid and higher) and were comparable to the controls in the recovery period.

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. 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).
Recovery 5 rats/sex	Microscopically: slight renal tubular Karyomegaly and pigment accumulation, mild typhlitis (wk 43).

Dose levels & groups	Major drug-related findings
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.

Comments: A NOAEL for oral bis-POCPMPA in rats could not be identified; it should be considered less than 30 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 4.87 mg/kg/day.

In rats, evidence of bone toxicity was seen at the high dosages (300 and 1000 mg/kg/day). With regard to the bone toxicity, a dosage of 100 mg/kg/day may be considered the NOEL. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 16.23 mg/kg/day (974 mg/day for a 60 kg person).

In monkeys (15/19), bone toxicities were seen after > 10 months of daily dosing with sc PMPA at dose level of 30 mg/kg/day (Submission No. 009). When treatment was initiated to dams during the second trimester, bone toxicities were seen at 2 and 7.5 months of age in 2/9 affected neonates. The AUC values in the monkeys undergoing long term PMPA treatment at 30 mg/kg/day ranged from 97.9 to 240 µg*hr/ml. In the clinic, oral bis-POCPMPA has been used at dose levels of 75, 150 or 300 mg/day; the AUC values were 0.76, 1.66 or 3.34 µg*hr/ml. Thus, there is a several folds safety margin in the clinic with respect to effects on bone.

Table 3
Estimated safety margins in rats

Target organ	Study duration	NOEL,MEL mg/kg/day	AUCss µg*hr/ml for NOEL, MEL	Margin relative to human AUCss
GI toxicity	42-week	30, 100	4, 8	1 time, 3 times
Renal toxicity	42-week	300, 1000	18, 65	6 times, 20 times
Bone mineral loss	42-week	100, 300	8, 18	3 times, 6 times

MEL = minimal effect level

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

8. Five day repeated dose oral toxicity study of bis-POCPMPA in beagle dogs, Lot # 1156-27-13, Corning Hazelton Wisconsin, Inc., Madison, WI, November 8, 1996, (96-TOX-4331-05/TA4331-00002)

Groups of male beagle dogs (2/group) were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 9 (low), 45 (mid) or 180 mg/kg/day (high) once daily for 5 days. Blood samples were collected at 0, 0.5, 1, 2, 4, 8 and 24 hr postdose on day 5. Results: no adverse findings were noted in male dogs (low). Drug-related findings included (mid) non-formed, mucoid, liquid, few or no feces; vomitus; low food consumption; loss of body weight (average loss was 0.4 kg); and increased serum creatinine levels. Drug-related findings included (high) few, no, liquid and mucoid feces; vomitus; low food consumption; and loss of body weight (average loss was 1.1 kg). Oral administration of bis-POCPMPA (high) was associated with renal failure in 1/2 dogs; abnormal results included markedly increased urea nitrogen, creatinine, inorganic phosphorus and potassium values, and decreased calcium and chloride values. Macroscopic findings related to drug were restricted to edema in the abdominal cavity for 1/2 dogs (high). Histopathologic findings: related to drug were present in bone marrow (moderately hypo-cellular), esophagus (ulceration and supportive inflammation), large and small intestines (slight to moderate crypt epithelial necrosis), kidney (slight to moderately-severe tubular degeneration and slight tubular regeneration), lymphoid tissue of the spleen, gut-associated lymphoid tissue (GALT), and submandibular and mesenteric lymph nodes (high). Drug-related change was minimal renal tubular degeneration with individual epithelial cell necrosis in both animals (mid).

Comments: The NOEL for oral bis-POCPMPA in male dogs was 9 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 4.86 mg/kg/day.

9. Four week oral gavage toxicity study of bis-POCPMPA in the beagle dog, Lot # TX4331-96-02, Clinical Trials Bioresearch, Sennebille, Quebec, February 24, 1997, (96-TOX-4331-004)*

Groups of male and female beagle dogs (4/sex/group) 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 4 weeks. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose on days 1 and 28. Results: no adverse findings were noted in male and female dogs (low). Drug-related decreases in body weight (13%) and food consumption (7%) were seen in male and female dogs (high). Treatment-related decreases in red blood cell counts, hemoglobin concentration and hematocrit were seen in male and female dogs (mid and high) and in the mean corpuscular hemoglobin concentration of female dogs (high). Treatment-related increases in blood urea nitrogen and creatinine levels were seen in 1/4 males (high). A statistically significant increase in total bilirubin was seen in 3/4 males and 3/4 females (high) and in 2/4 females (mid). A decrease in cholesterol levels was seen in 2/4 males and 2/4 females (mid) and in all males and 2/4 females (high). Histopathologic findings: drug-related changes were seen in the kidneys of male and female dogs (mid and high). The lesions were described as tubular degeneration/regeneration and tubular karyomegaly with an apparent dose-related increase in severity.

Comments: The NOEL for oral bis-POCPMPA in male dogs was 3 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 1.62 mg/kg/day.

10. Four week oral gavage toxicity study of bis-POCPMPA in the beagle dog, Lot # 2454-A-1P, Clinical Trials Bioresearch, Sennebille, Quebec, September 15, 1999, (98-TOX-4331-003)

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. A complete histopathologic examination was performed on the following tissues: abnormalities, tissue masses, adrenals, aorta (thoracic), bone marrow smear (prepared from femur), brain

(cerebellum, cerebrum, medulla), cervix, esophagus, eyes, femur (with stifle joint and marrow), heart, intestine-large (cecum, colon, rectum), intestine-small (duodenum with pancreas, jejunum, ileum), kidneys, liver, liver with bronchi, lymph nodes (bronchial, mandibular, mesenteric), ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (biceps femoris), skin with mammary gland, spinal cord (thoracolumbar), spleen, sternebra with marrow, stomach (cardia, fundus, pylorus), testes with epididymides, thymus, thyroid gland (with parathyroid glands), tongue, trachea, urinary bladder, uterus and vagina. Results: 2/4 dogs (very high) became moribund and were euthanized on day 5 or 6 after receiving only one dose on day 1. Signs of moribundity (ie, lack of righting and pinch reflexes, decreased muscle tone, lying on side, deep breathing, eye partly closed and/or weakness) and blood in the feces were observed in the dogs (very high) that were euthanized. Clinical signs: of toxicity included vomitus (very high) and abnormal feces (colored/mucoid/soft/liquid) in all dose groups; thinness, dehydration and salivation (mid, high and very high); and cold to touch and decreased feces and activity (high and very high). Body weights and food consumption: transient body weight losses and decreased body weight gains occurred in all dose groups to about the same degree of change. Notable reductions in food consumption were recorded in the high and very high dose groups. No changes were recorded in hematological parameters. Clinical Chemistry: marginal decreases in cholesterol (mid) and increases in cholesterol were observed in dogs (high and very high), and marginal to slight increases in creatinine, total bilirubin, triglycerides, and increased urine volume were observed in all dose groups on day 29. For the two dogs euthanized moribund, changes in clinical chemistry parameters on day 5 or 6 were mainly due to combination of kidney toxicity, dehydration and multiple episodes of vomiting. These changes included markedly increased BUN, creatinine, creatine kinase, total protein, potassium, calcium and phosphorus values; slight to moderate increases in total bilirubin, AST, ALT, albumin, globulin and cholesterol values; sight to moderate decreases in glucose, sodium, chloride and bicarbonate values. Decreased urine pH and increased urine protein were also noted in one of these dogs. Gross and histopathologic findings: macroscopic finding recorded at necropsy considered to be drug related included pale kidneys (all treated animals). Other findings such as gastric changes and dark areas on the tongue and gums were recorded (very high). Histopathological examination performed on day 29 revealed slight to moderate drug-related changes in the kidney and included renal tubular karyomegaly, degeneration/regeneration, single cell necrosis and focal to multi focal mononuclear cell infiltration (all treated animals). Lesions were present with no evidence of a difference in severity between groups. The severity of the renal lesions in the dogs

(very high) euthanized early on were more moderate to severe, described as tubular diffuse necrosis and tubular mineralization.

Comments: Greater toxicity including deaths were observed when drug was administered as a single dose of 210 mg/kg. No other obvious differences in this study were observed in the effects produced by drug in beagle dogs on a daily, twice daily, or twice weekly dose schedule. A NOEL for oral bis-POCPMPA in dogs could not be identified.

Previously, the sponsor conducted a 4-week toxicity study of bis-POCPMPA in dogs (96-TOX-4331-004) where groups of male and female beagle dogs (4/sex/group) 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 4 weeks. The NOEL for oral bis-POCPMPA in male dogs was 3 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 1.62 mg/kg/day.

11. Interim (13-week portion) report: a 13 and 42-week oral gavage toxicity study (with a 13-week recovery period) of bis-POCPMPA in the beagle dog, Lot # LH0424, Clinical Trials Bioresearch, Sennebille, Quebec, Canada March 10, 1998, (97-TOX-4331-001)

Groups of male and female beagle dogs (4/sex/group) 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 or 42 consecutive weeks. Data from animals in the 42-week cohort for this study will be reported separately. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose on days 1 and 90. Results: there were no treatment-related clinical signs of toxicity, no effects on food consumption and ophthalmological or electrocardiographic findings. There were no treatment-related hematological effects observed. Body weights: there was an initial body weight loss in the males (mid and high) followed by body weight gains in these groups similar to the vehicle group for the remainder of the 13-week dosing period. Terminal body weights were slightly and non-significantly decreased in males (mid and high) relative to the controls. Biochemical parameters: drug-related and statistically significant changes included: marginal increases in bilirubin, creatinine and chloride (high) at week 6; marginal or slight increases in total bilirubin, creatinine, chloride, AST, ALP levels (mid and high) at week 13; and decreased potassium and glucose levels (high) at week 13. A marked presence of glucose in urine was observed (high) at week 6 and 13. Organ weights: although not statistically significant, mean kidney weights (absolute and relative) were higher in animals (high) than the controls. Macroscopic findings: pale discoloration of the renal cortex was observed in all high dose

male and female dogs. Histopathologic findings: drug-related changes were seen in the kidneys of male and female dogs (mid and high) and male dogs (low; 2/4). The lesions were graded from slight to mild and characterized by one or more combination of the following diagnoses: tubular karyomegaly, dilatation or degeneration/regeneration and chronic interstitial nephritis. The latter three lesions were only observed in the high dose animals while tubular karyomegaly was seen in all treatment groups. There was an apparent dose-related increase in the incidence and/or severity for tubular karyomegaly. Chronic interstitial nephritis characterized as histologically interstitial fibrosis and mononuclear cell infiltration was recorded in two high dose males. The testicular hypo/aspermatogenesis and associated epididymidal oligo/aspermia was linked to sexual immaturity.

Comments: A NOAEL for oral bis-POCPMPA in dogs could not be identified; it should be considered less than 3 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 97.29 mg/kg/day.

12. A 13 and 42-week oral gavage toxicity study (with a 13-week recovery period) of bis-POCPMPA in the beagle dog, Lot # LH0424, Clinical Trials Bioresearch, Sennebille, Quebec, Canada, February 4, 1999, (97-TOX-4331-001)

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. The following organs were dissected free of fat and weighted: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary, prostate, spleen, thymus, thyroid lobes and parathyroid glands, and uterus. A complete histopathologic examination was performed on the following tissues: adrenals, aorta (thoracic), bone marrow smear (prepared from femur), brain (cerebellum, cerebrum, medulla), cervix, esophagus, eyes (with optic nerve and Harderian gland), femur (with stifle joint and marrow), heart, intestine-large (cecum, colon, rectum), intestine-small (duodenum with pancreas, jejunum, ileum with GALT), kidneys, liver, liver with bronchi, lymph nodes (bronchial, mandibular, mesenteric), ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (biceps femoris), skin with mammary gland, spinal cord (thoracolumbar), spleen, sternebra with marrow, stomach (cardia, fundus, pylorus), testes with epididymides, thymus, thyroid gland (with parathyroid

glands), tongue, trachea, urinary bladder, uterus, vagina, and vertebrae. Results: there was one death (control male) during the treatment period; the likely cause of death was a fibrinohemorrhagic bronchopneumonia possibly caused by a gavage accident. There were no treatment-related clinical signs of toxicity, no effects on food consumption and ophthalmological or electrocardiographic findings. Body weight: loss was observed in animals (mid males during the first week) and high dose group animals during the first two weeks of dosing. Although, subsequent weight gains appeared to be similar to the vehicle control group, mean values for these groups remained marginally lower throughout the remainder of the dosing and recovery period. Biochemical parameters: drug-related changes included slightly increased total bilirubin and chloride concentrations, alkaline phosphatase and creatine kinase activities, and slight to moderate decreases in cholesterol, bicarbonate and potassium concentrations. Potassium, chloride, total bilirubin and alkaline phosphatase levels showed a partial return to normal or evidence of recovery, after the recovery period. Minimal drug-related increased changes in creatinine and glucose (week 13) values were observed. Organ weights: absolute and relative kidneys weights (high) were generally higher than controls at both necropsy examinations (weeks 14 and 42). Gross and histopathologic findings: drug-related gross lesions were observed in the kidneys (mid and high). Gross examination revealed pale discoloration of the renal cortex (mid, 1/4 ♂, 13-week) and all animals (mid and high; 13-week and 42-week). Both (2/2) males (mid, recovery) and 1/2 males and 2/2 females (high, recovery) showed pale kidneys. With an apparent dose-related increase, tubular karyomegaly was noted in treated groups; dilatation or degeneration/regeneration and interstitial nephritis were usually observed in animals (mid and high) at weeks 14, 42 and 55. Tubular karyomegaly was seen in animals (low, 13- and 42-week). Interstitial fibrosis and mononuclear cell infiltration characterized as chronic interstitial nephritis was observed (mid, recovery) and high dose group animals throughout the study with highest occurrence in the recovery animals. Tubular karyomegaly, dilatation and degeneration/regeneration were present in the recovery animals but showed a lesser severity than the main study animals. In the liver, centrolobular sinusoidal dilatation/congestion and/or slight centrolobular sinusoidal cell pigment accumulation were observed (high) at all necropsies, with the highest occurrence in the main study animals at week 42 and the lowest occurrence in the recovery animals at week 55. Bone evaluation: marginal/slight decreases were observed in bone mineral content and bone mineral density of the slice and trabecular and cortical areas of the distal femur metaphysis of males and females (high) for 13 or 42 weeks, with some evidence of recovery at week 55. Decreases in cortical thickness generally correlated with slight decreases in periosteal circumference and slight increases in endosteal

circumference. Minimal effects on the cortex were observed in the mid-femur diaphysis. Statistically significant decreases in cortical thickness and decreases in endosteal circumference were observed for females (high) at week 42. Cortical area and bone mineral content were statistically significantly decreased at week 42 for males (high). Changes in tested biochemical markers of bone turnover (increased urinary N-telopeptide, increased urinary calcium and phosphorus, increased bone specific alkaline phosphatase and decreased 1,2,5-dihydroxy-vitamin D_3) were consistent with bone activation and resultant effects on peripheral quantitative computed tomography (pQCT)-derived parameters.

Comments: A NOAEL for oral bis-POCPMPA in dogs could not be identified; it should be considered less than 3 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 1.62 mg/kg/day.

Evidence of bone toxicity in the dogs was seen at the high dosage (30 mg/kg/day). With regard to the bone toxicity, a dosage of 10 mg/kg/day may be considered the NOEL. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 16.21 mg/kg/day (973 mg/day for a 60 kg person).

Table 4
Estimated safety margins in dogs

Target organ	Study duration	NOEL,MEL mg/kg/day	AUCss µg*hr/ml for NOEL, MEL	Margin relative to human AUCss
GI toxicity	42-week	30	10	10 times
Renal toxicity	42-week	3, 10	2, 7	1 times, 2 times
Bone mineral loss	42-week	10, 30	7, 30	2 times, 10 times

MEL = minimal effect level

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

Single and multiple dose toxicology-Tenofovir

1. Single dose intravenous toxicity study of PMPA in Sprague-Dawley rats, Lot # 968-31-30, Gilead Science, Inc., Foster City, CA, May 30, 1996, (95-TOX-1278-009)

Groups of male Sprague-Dawley rats (3/group) received single iv bolus injections of PMPA. The study design is shown in Table 5.

Results: unscheduled deaths were 3/3 (#1), 3/3 (#2), 3/3 (#3) and 1/3 (#4) within 24-48 hr post-dose. Clinical signs of toxicity observed in all animals within 1 hr post-dose and included hypoactivity and soft or loose stool. Conclusion: the minimum lethal dose for PMPA could not be identified in the study; it should be considered less than 75 mg/kg. Based on a body surface conversion factor, an equivalent dose in humans would be less than 10.7 mg/kg.

Table 5
Study design

Group #	Dose level	Dose conc.	Dose volume	Route
1	100 mg/kg	10 mg/ml	10 ml/kg	iv bolus
2	100 mg/kg	20 mg/ml	5 ml/kg	iv bolus
3	100 mg/kg	60 mg/ml	1.76 ml/kg	iv bolus
4	75 mg/kg	15 mg/ml	5 ml/kg	iv bolus

2. Fourteen day intravenous infusion toxicity and toxicokinetics study of PMPA in Sprague-Dawley rats, Lot # RDE-834-169, Corning Hazelton Wisconsin, Inc., Madison, WI, July 15, 1996, (95-TOX-1278-003/CHW 6511-112)*

Groups of male and female Sprague-Dawley rats (10 animals/sex/group) received iv infusion (over 60 min period) of PMPA at dose levels of 0 (vehicle control), 5 (low), 25 (mid) or 100 mg/kg/day (high) once daily for 14 days. Each treated group consisted a separate toxicokinetics arm (16 males/group). For toxicokinetic analyses, blood samples were taken at 0, 5, 15 and 30 min and 1, 2, 4 and 6 hr post-dose. Results: on day 6, one female (control) died during dose administration. Macroscopic examination revealed that the death of this animals was due to an atrial thrombus of the heart; the thrombus was caused by the catheter. Clinical signs: included non-formed and liquid feces (high). These observations were noted for some high dose animals during or within 4 hr post-dose on days 1 and 2. These findings were not observed for any of the animals after day 2. There were no notable differences in body weights, body weight gains or food consumption attributed to the test material. Clinical chemistry: males at all dose levels had lower total protein, albumin, calcium, inorganic phosphorus and sodium. Microscopic findings: there were no drug-related microscopic findings. Microscopic findings associated with the iv catheters were similar for control and high dose groups animals. The most frequent findings were fibrosis with inflammation at the catheter exit sites, fibrosis with granulomatous inflammation at the catheter entrance sites and thrombi, and intimal hyperplasia at the catheter tip. Except for the control female that died during the study, none of

the lesions associated with the catheters were severe enough to affect the general health of the animals. Plasma pharmacokinetics: parameters are shown in Table 6. Following the first iv infusion, concentrations of PMPA in plasma declined in a bi-exponential manner. The pharmacokinetics of PMPA in rats appeared to be dose-dependent.

Table 6
Pharmacokinetic parameters of PMPA following repeated daily iv infusion in Sprague-Dawley rats

Parameter	Dose (mg/kg/day)					
	5		25		100	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Cmax (µg/ml)	1.44	1.43	10.4	8.26	44.3	69.6
T½ (hr)	1.56	1.71	3.58	3.91	2.25	1.84
AUC_{∞} (µg*hr/ml)	1.45	1.54	10.5	9.67	46.6	74.1
MRT (hr)	1.78	1.85	2.16	2.28	1.62	1.54
CL (l/hr/kg)	3.46	3.25	2.38	2.59	2.15	1.35
Vdss (l/kg)	6.15	6.02	5.15	5.91	3.47	2.08

Comments: A target organ could not be identified in the study. The NOEL for iv PMPA in male and female rats was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of iv infusion of PMPA in humans would be 14.3 mg/kg/day.

3. Fourteen day intravenous infusion toxicity study of PMPA in Cynomolgus monkeys, Lot # H601, Sierra Biomedical, Inc., Sparks, NV, August 8, 1996, (96-TOX-1278-002/SBI 0419-11)*

Groups of male and female Cynomolgus monkeys (4/sex/group) received one hr iv infusions of PMPA at dose levels of 0 (vehicle control), 3 (low), 10 (mid) or 25 mg/kg/day (high) once daily for 14 consecutive days. Results: no animal died during the course of the study. No treatment-related clinical signs were evident. No treatment-related effect on body weight, body weight gain or food consumption was noted. EEG recording and ophthalmic examinations were normal. Organ weights: mean spleen/body weight was slightly higher (2 and 15%, respectively) in mid and high dose groups.

Mean thymus weight (both absolute and relative to body weight) was lower (low and mid) than control group. Gross necropsy: a dose-related increase in the incidence of parovarian cysts of the ovary was noted in the female animals. Histologic examination of the ovaries confirmed the presence of cysts located external to the ovarian capsule. The increase in ovary weight (both absolute and relative to body weight) correlated with the occurrence of parovarian cysts. Histopathology: treatment-related finding were confined to the kidneys of high-dose animals. The renal alteration consisted of subtle degenerative changes in the kidney of some of the animals (high). In 2/4 males (high), the kidneys were characterized by mild multifocal tubular degeneration of the proximal convoluted tubules. Many of the degenerative tubules contained large basophilic epithelial cells that were frequently 3-5 times the size of normal epithelial cells (termed "megalocytosis"). Many of these cells contained eosinophilic intranuclear inclusions. Mitotic figures in these tubules were common. The kidneys from the other two males (high) showed no treatment-related alterations. Three females (high) had minimal degenerative changes of the proximal convoluted tubules. Alterations noted at the injection site (control, low, mid or high) consisted of minimal to mild fibrosis, inflammation, hemorrhage and myofiber, and collagen degeneration. Females (high) had a slightly increased incidence of these changes as compared to the controls.

Comments: Based on the results of the present study, kidney and injection sites were identified as the target organs. Other than the lesions at the injection site, the NOEL for PMPA administered to Cynomolgus monkeys as a one hr iv infusion once daily for 14 days was 10 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of iv PMPA in humans would be 3.3 mg/kg/day.

Bone toxicology-related Tenofovir and Tenofovir DF

1. A 3-day oral repeat dose study to evaluate serum and urine phosphorus levels in male rats treated with tenofovir DF and supplemented, following the final dose with ip or oral phosphate, Lot # 2454-A-2P, Gilead Sciences, Inc., Boulder CO, April 19, 2001, (R2000095)

Groups of male Sprague-Dawley rats (6/group) were administered either 0 or 400 mg/kg tenofovir DF by oral gavage on day 0, 1 and 2 to study whether or not serum and/or urine phosphorus levels were changed by supplementation with ip or oral phosphate immediately following the final dose. On day 2, the animals received neutral sodium phosphate by either oral gavage or ip injection (280 mg/kg phosphate). Urine and blood samples were collected. The animals were euthanized on day 3. Results: no gross toxicities were observed. On day 2 prior to phosphate

supplementation, rats treated with tenofovir DF (400 mg/kg) had statistically significantly lower serum and urinary phosphorus concentrations compared to the control animals. On day 2 at 4 hr post phosphate supplementation, all groups had greater urinary phosphorus concentrations compared to the pre-supplemental concentrations. Tenofovir DF treated animals supplemented with ip phosphate had an equivalent rise in urinary phosphorus compared to the controls administered ip phosphate. However, tenofovir DF treated animals supplemented with oral phosphate had approximately half the increase in urinary phosphorus concentrations compared to the controls. The tenofovir DF treated group had increases in serum phosphorous concentration between 10% and 18% at 4 hr post phosphate supplementation compared to serum phosphorus prior to phosphate supplementation. By 24 hr post phosphate administration, urinary and serum phosphorus values for all groups were approaching pre-phosphate supplementation levels. Conclusions: the administration of tenofovir DF (400 mg/kg/day) caused a marked hypophosphaturia and a slight reduction of the serum phosphorus concentration. The administration of phosphate by either oral gavage or ip injection resulted in phosphaturia that was greater by the latter route of administration. The findings suggested that orally administered tenofovir DF impaired intestinal phosphate absorption which could be overcome by phosphate supplementation administered either orally or ip. Orally administered tenofovir DF did not appear to alter the function of the kidney to appropriately reabsorb or excrete phosphate.

2. A 3-day oral or iv repeat dose study to evaluate serum and urine phosphate concentrations in male rats treated with tenofovir or tenofovir DF and supplemented with oral phosphate, Lot # H901, 2454-A-2P, Gilead Sciences, Inc., Boulder CO, April 20, 2001, (R2000099).

Groups of male Sprague-Dawley rats (6/group) were administered either 50 mM citric acid by oral gavage (controls), 50 mg/kg tenofovir by iv, 180 mg/kg tenofovir by oral gavage, 400 mg/kg tenofovir DF by oral gavage on day 0, 1 and 2 to study whether or not serum phosphorus concentration and/or urine phosphate excretion in the rat treated with iv/po tenofovir or tenofovir DF by oral gavage were changed by supplementation with oral phosphate immediately following the final dose. On day 2, the animals received neutral sodium phosphate by oral gavage (280 mg/kg phosphate). Urine and blood samples were collected. The animals were euthanized on day 3. Results: no gross toxicities were observed. Histological examination revealed no treatment related changes in tissues from animals given tenofovir either by iv or oral routes. Tenofovir DF treated rats had minimal renal proximal tubular epithelial karyomegaly and duodenal epithelial hyperplasia and inflammation. Rats treated with tenofovir DF had

statistically significantly less urinary phosphorus than control animals on days 1 and 2. On day 2, following phosphate supplementation, control animals increased urinary excretion of phosphorus by approximately 638% over a 4 hr period. Tenofovir treated rats had increases in urinary phosphorus between approximately 620% to 804%. Tenofovir DF treated animals supplemented with phosphate had an increased in urinary phosphorus excretion of approximately 1400% over the same period but overall excretion remained one-third the rate of control animals. The tenofovir DF treated group had an increase in serum phosphorus of approximately 23% at 4 hr pos-phosphate supplementation compared to serum phosphorus prior to phosphate supplementation. The iv tenofovir treated group had an increase in serum phosphorus of approximately 6%, which was equivalent to the increased for control and oral tenofovir treated groups. By 24 hr post-phosphate administration, urinary phosphorus concentration for all groups had declined and serum phosphorus concentrations approached pre-phosphate supplementation concentrations. Rats treated with 400 mg/kg/day tenofovir DF for two days had marked hypophosphaturia. Rats treated with oral tenofovir did not have any alteration in serum or urinary phosphorus concentrations prior to phosphate supplementation. Rats treated with iv tenofovir had decreased serum phosphorus on days 1 and 2 and no changes in urinary phosphorus. Oral supplementation with phosphate to tenofovir DF treated rats increased serum and urinary phosphorus concentrations. Conclusions: the findings suggested that tenofovir DF, but not tenofovir, the active moiety of the pro-drug, impaired intestinal phosphate absorption. Oral phosphate supplementation overcame the impairment.

3. A 28-day study to evaluate the effects of bis-POCPMPA on bone following daily administration by gavage in the Sprague-Dawley rat, 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: two male rats (one control and one high) were found dead on day 17. The cause of death was not established based upon post mortem examination. Clinical observations: a number of treated animals were noted to demonstrate ptyalism pre and post dosing. The ptyalism was most marked in the high dose animals and persisted throughout the

treatment. Body weight gain: was significantly reduced in the high dose animals. Biochemical markers of bone turnover and associated parameters: significantly elevated deoxypyridinoline levels, a marker of bone resorption, were observed for the high dose animals. Statistically significant increased serum calcium, decreased urinary phosphorus values and a decrease in 1,2,5-dihydroxycholcalciferol were observed for the high dose animals at the end of treatment period compared to the controls. An increase in urinary calcium was also noted in the high dose group; however, the difference did not attain statistical significance. No effect on PTH was observed. Treatment-related effects were noted on pQCT-derived bone mineral content and bone mineral density of the distal femur metaphysis and/or diaphysis. Microscopic observations: renal cortical tubular karyomegaly was the sole finding ascribed to the treatment. This observation was seen in the high dose animals (incidence 90%) and was graded minimal in severity. No histological changes attributed to the treatment were found in the decalcified sections of distal femur. Histomorphometric evaluation of the metaphysis of the tibia revealed an increased bone resorption along the trabecular surfaces in animals treated with tenofovir (low or high) as measured with osteoclast surface. Bone formation and mineralization remained unaffected by the treatment.

Comments: Daily oral gavage administration of tenofovir to male rats for 28 days resulted in treatment related effects at dose levels of 40 or 400 mg/kg/day, most marked at the high dose level. For the high dose group, treatment related clinical signs, reduced body weight and body weight gains, effects on pQCT-derived bone mineral content and bone mineral density of the distal femur metaphysis and/or diaphysis, and effects on biochemical markers of bone turnover were noted.

4. Summary report: PMPA in SIV-infected and uninfected rhesus monkey: Studies from Martin and Tsai Labs, April, 23, 2001, (T1278-00034)

Nonclinical antiviral efficacy studies of PMPA in SIV-infected and noninfected rhesus monkey were carried out at the California Regional Primate Research Center and the Tulane Regional Primate Research Center. The sponsor has provided preliminary summary reports of PMPA-associated bone lesions in the monkey. Bone toxicities were seen in the monkeys (15/19) after > 10 months of daily dosing with sc PMPA at dose level of 30 mg/kg/day. When treatment was initiated to dams during the second trimester, bone toxicities were seen at 2 and 7.5 months of age in 2/9 affected neonates dosed throughout the study with 30 mg/kg/day. The bone toxicity was characterized variably as abnormal growth plates and trabecula of the ribs and femurs, bone deformities and displacements, rib fractures, decreased bone densities, joint

swellings and bone loss in the spine or pelvis. Elevated ALP and decreased serum phosphorus levels were observed in the animals with bone lesions; calcium values were normal. SIV infection did not appear to influence the incidence or severity of bone lesions. Histopathology: showed diffuse and marked hyperplasia and dysplasia (thickened, wavy and undulating) of the femur (distal) growth plate, trabecular hypertrophy of the femur (distal) with moderately to markedly widened osteoid seams and a few trabeculae subjacent to the growth plate of the vertebral column having cartilaginous cores. Plasma concentrations: of PMPA generally reached a maximum at 30 min postdose and declined thereafter in a bi-exponential manner. The AUC values in the monkeys undergoing long term PMPA treatment at 30 mg/kg/day ranged from 97.9 to 240 µg*hr/ml.

Comments: Preliminary data from ongoing antiviral efficacy studies with sc PMPA (30 mg/kg/day) demonstrated bone abnormalities in SIV-infected and noninfected rhesus monkeys. No evidence for bone lesions similar to what has been seen in the primates studies has been seen in PMPA or PMPA prodrug studies in other animals species or humans to date (ie, no clinical sign, alterations in clinical chemistries or histological evidence for bone abnormalities). The sponsor postulated that bone toxicities in monkeys might relate to the magnitude of dose, duration of treatment, extent of systemic exposure, tissue distribution or species specific factors. Studies to further evaluate the relationship of the effect on bones to species, age, dose/systemic exposure, duration of therapy and to evaluate reversibility are ongoing. The sponsor is collecting blood, urine and bones from the ongoing 13- and 26-week oral toxicity studies of PMPA prodrug in rats and dogs. The sponsor will be modifying the clinical development plans as appropriate to ensure safety based on data provided from the ongoing nonclinical studies.

PMPA-related bone toxicity update:

The summary reports (Submission # 9) indicated that bone lesions were seen in the monkeys (15/19) after > 10 months of daily dosing with sc PMPA at dose level of 30 mg/kg/day. When treatment was initiated to dams during the second trimester, bone toxicities were seen at 2 and 7.5 months of age in 2/9 affected neonates. PMPA-related bone lesions in the monkeys (30 mg/kg/day, sc) were characterized variably as abnormal growth plates and trabecula of the ribs and femurs, bone deformities and displacements, rib fractures, decreased bone densities, joint swellings and bone loss in the spine or pelvis. Elevated ALP and decreased serum phosphorus levels were observed in the animals with bone lesions; calcium values were normal. SIV infection did not appear to influence the incidence or severity of bone lesions. Histopathology: showed diffuse and marked hyperplasia

and dysplasia (thickened, wavy and undulating) of the femur (distal) growth plate, trabecular hypertrophy of the femur (distal) with moderately to markedly widened osteoid seams and a few trabeculae subjacent to the growth plate of the vertebral column having cartilaginous cores. Plasma concentrations: of PMPA generally reached a maximum at 30 min postdose and declined thereafter in a bi-exponential manner. The AUC values in the monkeys undergoing long term PMPA treatment at 30 mg/kg/day ranged from 97.9 to 240 µg*hr/ml.

Based on bone changes in the monkeys, the ongoing protocols for chronic toxicology studies of PMPA in rats and dogs (13/42-week) were revised to incorporate the evaluations of biochemical markers of bone metabolism and bone morphometry to provide possible evidence of toxicity to bone in the animals treated with PMPA prodrug.

Summary of toxicity reports: Studies of Martin (Dr. Taylor)

Three groups of rhesus monkeys (5/group) were dosed for 28 days with PMPA (30 mg/kg/day, sc) starting at either 24 hr (group 2), 7 days (group 3) or 14 days (group 4) post infection with SIV. An additional group of monkeys was dosed for at least 434 days (group 5) with PMPA (30 mg/kg/day, sc) starting a 14 days post SIV infection. The vehicle control (group 1) was dosed with saline (sc) for 28 days starting at 24 hr post SIV infection.
Results:
no behavioral signs indicative of bone toxicities (eg, reluctance to stand, abnormal spine curvature) were observed in monkeys (Groups 2-4). Alterations in clinical chemistry parameters (group 4) on day 364 were indicative of a treatment effect on bone. Compared to the vehicle control group, mean serum phosphorus level was reduced (48%) and mean ALP activity was increased (650%); serum calcium levels remained within the normal range for the monkeys. Bone evaluations on day 364 (group 5): one of the 5 monkeys had decreased bone density (L3-L5) and bone displacement (T4-T5) on X-ray examination, with normal patellar and triceps reflexes and no loss of muscle tone or atrophy. Certain changes in distal vertebrae may represent weight bearing stress-induced changes. The other 4 monkeys (group 5) had some bone loss in the spine or pelvis.

Table 7
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 8

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

Lately, the 13/42-week chronic toxicology of studies of PMPA Prodrug in rats and dogs have been reviewed in the division (Submission # 045). Conclusions: the peripheral quantitative computed tomography (pQCT) evaluations of distal femur metaphysis and mid-femur diaphysis and biochemical bone marker data demonstrated dose-dependent toxicity to bones in both rats and dogs after 13 and 42 weeks of the treatment. Bone changes characterized by decreases in bone mineral content, bone mineral density, thickness and periosteal circumferences, and increases in the endosteal circumference, were consistent with a PMPA Prodrug-related increase in bone resorption for both the test species. Urinary markers of bone resorption were also increased in rats (deoxypyridinoline) and dogs (N-telopeptide), providing confirmatory evidence for a drug-related effect on bone.

In rats, evidence of bone toxicity was seen at the high dosages (300 and 1000 mg/kg/day). With regard to the bone toxicity, a dosage of 100 mg/kg/day may be considered the NOEL. Based on a body surface conversion factor, an equivalent dose of oral bis-PMPA Prodrug in humans would be 16.23 mg/kg/day (974 mg/day for a 60 kg person).

Evidence of bone toxicity in the dogs was seen at the high dosage (30 mg/kg/day). With regard to the bone toxicity, a dosage of 10 mg/kg/day may be considered the NOEL. Based on a body surface conversion factor, an equivalent dose of oral bis-PMPA Prodrug in humans would be 5.4 mg/kg/day (324 mg/day for a 60 kg person).

In monkeys (15/19), bone toxicities were seen after > 10 months of daily dosing with sc PMPA at dose level of 30 mg/kg/day (Submission No. 009). The AUC values in the monkeys underwent long term PMPA treatment at 30 mg/kg/day ranged from 97.9 to 240 µg*hr/ml. When treatment was initiated to dams during the second trimester, bone toxicities were seen at 2 and 7.5 months of age in 2/9 affected neonates. AUC values in the pregnant females receiving sc PMPA at 30 mg/kg/day were 87-117 µg*hr/ml.

In the clinic, oral bis-PMPA Prodrug has been used at dose levels of 75, 150 or 300 mg/day; the AUC values were 0.76, 1.66 or 3.34 µg*hr/ml. Thus, there is a several fold safety margin in the clinic with respect to effects on bone.

Comments: Previously, preliminary data from ongoing antiviral efficacy studies with sc PMPA (30 mg/kg/day) demonstrated bone abnormalities in SIV-infected and noninfected rhesus monkeys. Based on the results, the sponsor was requested to conduct a 12-month toxicity study in rhesus monkeys to further characterize the bone toxicity. Additionally, Segment II and III reproductive toxicity studies in rhesus monkeys were recommended.

Based on results of the 13/42-week chronic toxicology studies of PMPA Prodrug in rats and dogs, the rat and dog appeared to be appropriate models for evaluating bone and related toxicities of PMPA Prodrug.

Results of a reproductive toxicology study of PMPA Prodrug (embryo-fetal development in rats) were reported previously (Submission # 031) and no adverse effects were noted. The sponsor has completed studies for effects on embryo-fetal development in rabbits and effects on fertility and early embryonic development in rats (final reports will be submitted in July 1999). A study for effects on pre- and postnatal development in rats was delayed pending the outcome of the bone evaluations (13/42-week chronic toxicology of studies of PMPA Prodrug in rats and dogs) and is currently scheduled to begin in July 1999.

5. Summary of PMPA toxicity data in rhesus monkey, Univ of California, Davis, CA, September 30, 2000, (P2000124)

When a range of PMPA doses (4-30 mg/kg, sc, once daily) were administered for a short period of time (ranging 1 day to 8 weeks) to 39 newborn or infant monkeys, no adverse events on their health or growth were observed; this included a subset of 12 animals which were monitored for more than 2 yr afterwards. In contrast, daily administration of a high dose of PMPA (30 mg/kg) for prolonged periods of time (>11-24 months) to 15 animals led to reduced clearance of PMPA and induced a Fanconi-like syndrome (proximal renal tubular disorder), with glucosuria and hypophosphatemia. In most animals, this process led to bone demineralization, growth plate deformations and growth restriction. These renal effects appeared to be largely reversible or alleviated following either complete withdrawal of PMPA treatment or reduction of the daily dosage regimen from 30 to 5-10 mg/kg. Finally, to evaluate the safety of a prolonged low dose treatment regimen, 3 newborn monkeys were started on a 10 mg/kg/day dosage regimen shortly after birth; these 3 animals are healthy and have normal renal function, bone density and growth after more than 2 yrs of treatment. Conclusions: these findings suggested that renal toxicity of PMPA and the resulting effect on bone density are restricted to prolonged high dose treatment regimens, while short term administration of relatively high dose and prolonged low dose administration showed little effect.

6. Conclusions report: Evaluation of radiographs from control and PMPA treated juvenile rhesus monkeys, April, 12, 2001, (P2000123)

All the films, the thoracic vertebrae and intervertebral spaces, ribs and sternebrae were evaluable. All films appeared to show normal skeletal conformation and development in these animals.

There were no radiographic signs of pathology or significant variation from normality attributable to PMPA treatment.

Addendum 1: Selected clinical pathology values and pharmacokinetics in 3 rhesus monkeys following the daily administration of PMPA at a dosage of 10 mg/kg by sc injection for 27 to 30 months.

Table 9
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$_{\infty}$ 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

7. Preliminary study: A 56-day study of tenofovir DF administered orally and of PMPA administered by sc injection to rhesus monkeys, Lot # H901, Sierra Biomedical, Inc., Sparks, NV, April 19, 2001, (P2000078)

Groups of male and female rhesus monkeys (3/sex/group) received a dose of the control article (50 mM citric acid solution, controls) or tenofovir DF orally at dose levels of 30 (low), 250 (mid) or 600 mg/kg/day (high), or PMPA via sc injection (30 mg/kg/day) once daily for 56 consecutive days. Due to the poor clinical condition of animals (high), this group was terminated early on days 20-23. Beginning on day 29, animals began to receive daily oral doses of a commercial phosphate supplement (125 mg/kg/day) to assess the general toxicity of tenofovir DF on calcium and phosphorus homeostasis and on bone remodeling (as assessed by biochemical markers). Results: clinical signs (tenofovir DF) consisted of an increased incidence of emesis and unformed stool (mid and high) and a suggestive increased incidence of low food consumption (high). Occurrence of decreased activity, hunched appearance and decreased body weights were observed in the high dose group that prompted the early termination of this group on day 20-23. Serum chemistry: a marked increased in serum BUN and creatinine concentrations in animals

(high). BUN and creatinine concentrations were slightly elevated in PMPA treated animals. Phosphorus levels tended to decline in most animals (low, mid and high) as well as PMPA treated animals. Other alterations were decreases in serum calcium (and plasma ionized calcium), electrolytes and protein, and decreases in LDH and bilirubin (high). Slight to moderate elevations of ALT and AST occurred in most animals (low, mid and high) and to a lesser extent in some PMPA treated animals. Hematology: modest decreases in the indicators of circulating red blood cell mass (RBC counts, hemoglobin and hematocrit in the mid, high and PMPA treated animals. Urinalysis: included an increase in specific gravity and a decrease in urine pH and protein in the high dose animals. The most salient finding was an increase in phosphorus excretion in the high dose animals. As expected, urine and serum phosphorus levels increased substantially in all animals during the period of oral phosphate supplementation. There also appeared to be an increase in urinary calcium/creatinine ratios in the high dose animals. Necropsy: included pale discoloration of the kidneys (mid and high or PMPA treated). The largest increases in kidney and liver weight occurred in the high dose animals. At day 57, kidney weight was increased in most of the animals (mid, high and PMPA treated). Histomorphologic changes: in the kidneys included tubular epithelial cells and consisted of karyomegaly, generalized swelling, fine cytoplasmic granulation and individual cell necrosis (mid, high and PMPA treated). Other ancillary changes affecting the tubules were seen less commonly, included thickening of basement membranes, intraluminal neutrophilic infiltration, tubular dilatation and intraluminal proteinaceous casts. Changes in the testes were evident in most of the males (low, mid, high and PMPA treated). These changes included degeneration of seminiferous tubules with interruption of spermatogenesis, often accompanied by the presence of spermatidic multinucleated giant cells. Thymic lymphoid atrophy was seen at an increased incidence and severity in animals (mid and high).

Comments: Based on the results of the present study, kidney, testes and thymic lymphoid were identified as the target organs. A NOEL could not be determined in this study. An increase in phosphorus excretion in the urine was clearly evident in the high dose animals. Oral phosphate supplementation during the second half of the study effectively restored normal serum phosphate levels despite continued administration of the test articles.

8. Conclusion report: Effect of PMPA treatment on cortical bone strength in rhesus monkeys, thesis, 2000, (T1278-00030)

Chronic treatment of PMPA (30 mg/kg/day) resulted in mineralization defects in developing and growing rhesus monkey cortical bone. Furthermore, the data suggested that reducing daily doses from 30 to 10 mg/kg/day or stopping treatment of a

period of time, can reverse the effects of PMPA on bone metabolism, resulting in normal, healthy bone. One half of the PMPA treated juvenile specimens had defective mineralization manifested by increased osteoid seam widths rather than increased numbers of osteoid seams, while all untreated juvenile specimens showed normal bone remodeling.

Among all PMPA treated animals, most of the adversely affected animals received daily treatments for periods varying between 4 months and 2 years, the entirety or greater part of their lives. The most severely affected animals received pre- and postnatal chronic PMPA treatments.

9. PMPA summary report: Human osteoblast calcium deposition in vitro (V2000122)

Human osteoblast-like cells (HOLC) were treated in the absence or presence of PMPA (10 µg/ml, a concentration approximately 3-times the human AUCss exposure, 300 mg/day dose) and maintained with twice weekly medium changes, for a 3-week period to determine whether PMPA adversely effects bone cells. Results: PMPA did not alter cellular calcium deposition, nor result in HOLC cytotoxicity.

Special toxicology-Tenofovir DF

1. A primary eye irritation study of bis-POCPMPA in rabbits (P4331-00022)

Six (3 male and 3 female) New Zealand White rabbits received a single application 0.076 g (0.1 ml) of bis-POCPMPA in the right eyes. Approximately 30 seconds later, the eyes of 3 rabbits were rinsed with sterile water (rinse group). The other 3 animals were not rinsed (no rinse group). The left eyes of both groups were untreated and served as the controls. The animals were examined for signs of irritation for up to 21 days following dosing. Results: no rinse group: drug produced corneal opacity in 3/3 test eyes at the 1 hr scoring interval. The corneal opacity did not resolve in any test eyes by day 21. Iritis was observed in 3/3 test eyes at 1 hr interval and resolved completely by day 21. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at 1 hr interval. The conjunctival irritation resolved in 2/3 eyes by study day 21. Rinse group: drug produced corneal opacity in 3/3 test eyes at the 1 hr scoring interval. The corneal opacity was resolved in 2/3 test eyes by day 21. Iritis was observed in 3/3 test eyes at 1 hr interval and resolved completely by day 21. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at 1 hr interval. The conjunctival irritation resolved in 2/3 eyes by study day 21. Conclusions: bis-POCPMPA was considered to be a very severe

irritant to the ocular tissue of the rabbit.

2. A primary skin irritation study of bis-POCPMPA in rabbits (P4331-00023)

Six male New Zealand White rabbits were evaluated for primary skin irritation with a single dermal application of bis-POCPMPA (0.5 g). The test article was moistened with deionized water and topically applied to the intact skin of the rabbits and left in place for 4 hr. Test sites were graded for erythema and edema at 24 and 72 hr after the single sample application. Results: under the conditions of this study, drug produced very slight erythema on 1/6 test sites at the 1 hr scoring interval. The dermal irritation resolved completely in the affected animals by 24 hr scoring interval. Conclusions: test article was considered to be a slight irritant to the rabbit skin.

3. A dermal sensitization study of bis-POCPMPA in guinea pigs (P4331-00024)

Ten male and female guinea pigs were topically treated with 75% bis-POCPMPA in polyethylene glycol 400 once per week to three consecutive weeks to evaluate the dermal sensitization potential of the test compound. Following a 2 week rest period, animals were challenged with 50% or 100% bis-POCPMPA. Results: following challenge with the test article group mean dermal scores were noted to be similar to the controls. Conclusions: test article was not considered to be a contact sensitizer in guinea pigs.

4. Bis-POC PMPA: Guinea pig ileum contractile response, Gilead Sciences, Inc., Boulder, CO, October 10, 2000, (T43331-00018/V2000009)

Guinea pig ileum was harvested and prepared for pre-incubation with bis-POC PMPA at dose levels of 0, 10, 30 or 100 µM for 5 min. The tissue was then exposed to agonists (acetylcholine, histamine or $BaCl_2$ to induce contraction. The percent inhibition was compared to 0 µM concentration. Results: the test compound (100 µM) significantly inhibited the contractile response up to 14% for each agonists tested. Conclusions: these results suggest that high concentration of bis-POC PMPA have direct inhibitory effect on smooth muscle contractility.

Reproductive toxicology-Tenofovir DF

1. Oral (gavage) fertility and general reproduction toxicity of bis-POCPMPA in rats, Lot # 2454-A-2P, Argus Research Labs., Horsham, PA, 10 June, 1999, (98-TOX-4331-001)

Groups of male rats [weight: 128-220 g; strain: Crl:CDBR

VAF/Plus; 25 animals/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high) once daily for 9 days. This study was terminated on day 10 of the study because of unexpectedly caused death or moribund sacrifice of 10 of 25 rats in the high dose group. Results: two male rats were found dead on day 7 or 8 and 8 male rats were moribund and sacrificed on day 8 or 9. The remaining 15 rats were sacrificed on day 10. Clinical observations: included statistically significant increased in the incidences of scant feces, red perioral substance, urine-stained abdominal fur, soft or liquid feces, excess salivation, no feces, emaciation and chromorhinorrhea (high). Body weight gain: was significantly reduced (mid) and significant body weight loss occurred (high) dose animals. Absolute and relative feed consumption values were significantly reduced for both mid and high. Necropsy: included distention of the stomach and intestines, fluid-filled intestines; raised, eroded and/or discolored areas on the mucosal and/or serosal surface of the stomach; and foci on the duodenum. Other gross lesions included a mass on the prostate and small prostate, seminal vesicles, kidneys, liver and/or spleen and large adrenals (high). Microscopic observations: changes in the stomach included ulceration and hyperplasia/hyperkeratosis of the squamous mucosa, necrosis of the glandular mucosa, erosions of the pyloric mucosa and glandular dilation (high, dead animals). Examination of the intestinal tract revealed severe damage characterized by ulceration, sloughing or erosions, hyperplasia and necrosis of the glandular epithelium and cystic/dilated glands. Variable degrees of atrophy were observed in the prostate, seminal vesicles and spleen in most of the rats examined (high). Conclusions: because of the unacceptable mortality and moribundity (high), the study was terminated.

2. Oral (gavage) fertility and general reproduction toxicity study of bis-POCPMPA in Sprague-Dawley rats, Lot # 2454-A-2P, Gilead Sciences, Inc., Foster City, CA, June 8, 1999, (98-TOX-4331-006)

Groups of male and female Sprague-Dawley rats (Crl:CD BR VAF/Plus; weights: 227-377 g; 25 rats/sex/group) were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 600 mg/kg/day (high) once daily (males) beginning 28 days before cohabitation and continuing through the day before sacrifice and to female rats once daily beginning 15 days before cohabitation and continuing through day 7 of presumed gestation. Results-male rats: significantly increased number of rats had excess salivation (low, mid or high) and the high dose animals had a dry, red perioral substance. No necropsy observations were considered drug-related. The weight of the paired kidneys and the ratios of the kidney weight to the terminal body weight were reduced and/or significantly reduced

(mid or high). Body weight gains were significantly ($p<0.01$) reduced (56.8%; high) on study days 1-8. This reduction resulted in the significantly reduced average body weight (high) on study day 8. Body weights were then significantly increased on study days 8-15 (high). After study day 15, body weight gains were comparable among the dosage groups. Reflecting the reduced body weight gains on study days 1-8, absolute (g/day) and relative (g/kg/day) feed consumption values (high) were significantly reduced on study days 1-8. All mating and fertility parameters were unaffected by the treatment. Female rats: significantly increased number of rats (mid or high) had a red or brown dry perioral substance, excess salivation and urine-stained abdominal fur during the precohabitation period. No necropsy observations were considered drug-related. Significant ($p<0.01$) body weight loss occurred (high) during study days 1-8, resulting in significantly reduced (36.25%) body weight gains on gestation days 5-8. Reflecting the reduced body weight gains on gestation days 1-8, absolute (g/day) and relative (g/kg/day) feed consumption values were significantly ($p<0.01$) reduced (high) on gestation day 1-15. Absolute and relative feed consumption values (mid or high) were significantly increased after gestation day 8. The number of rats with six or more consecutive days of diestrus during the precohabitation period was significantly increased, resulting in a significant decrease (high) in the number of estrous stages per 14 days. No Caesarean-sectioning or litter parameters were affected by the treatment. No gross external alterations (malformations or variations) were caused by the treatment.

Comments: On the basis of these data, the NOEL for oral bis-POCPMPA in the male rats was less than 100 mg/kg/day. The NOEL for the female rats was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than or equal to 16.23 mg/kg/day. The reproductive NOEL was 300 mg/kg/day; a human equivalent dose would be 48.7 mg/kg/day. The developmental NOEL was 600 mg/kg/day; a human equivalent dose would be 97.4 mg/kg/day.

3. Oral (gavage) developmental toxicity study of GS-4331-05 in rats, Lot # TX4331-97-03, Argus Research Lab, Horsham, PA, July 2, 1998, (97-TOX-4331-004)

Groups of presumed pregnant female Sprague-Dawley rats (strain: Crl:CD BR VAF/Plus; weight: 629-1125 g; 25 rats/group) were administered GS-4331-05 via oral gavage at dose levels of 0 (vehicle control), 50 (low), 150 (mid) or 450 mg/kg/day (high) on days 7-17 of presumed gestation. Dosages were selected based on the results from a dosage-range developmental toxicity study in rats, which showed mortality in one dam (1000 mg/kg/day), decreased maternal body weight and/or body weight gain (300 or

1000 mg/kg/day), decreased fetal body weights (1000 mg/kg/day) and maternal clinical signs of toxicity (1000 mg/kg/day). Results: all rats survived until scheduled sacrifice in the study. A statistically significant (p=0.05) increase in the incidence of localized alopecia on the underside occurred (high). Maternal body weights: GS-4331-05 caused a transient, statistically significant weight loss in dams (high) on gestation days 7-10. Body weight gains did not significantly differ among the 4 dosage groups throughout the remainder of the period. Reflecting the initial weight loss (high), body weight gain was significantly reduced for the entire period. Maternal feed consumption: GS-4331-05 significantly reduced absolute (g/day) and relative (g/kg/day) maternal feed consumption values on gestation days 7-12 (high); the absolute feed consumption value was also significantly reduced on gestation days 15-18. This effect of the test article was most severe on gestation days 7-10 and resulted in significant reductions in absolute and relative feed consumption values for the entire period. Caesarean-sectioning and litter observations: were unaffected by dosages of the test article. The litter averages for corpora lutea, implantations, live fetuses, total and early resorptions, percent live male fetuses (sex ratio), fetal body weights and percent resorbed conceptuses and the number of dams with any resorptions or with viable fetuses were comparable among the four dosage groups and did not significantly differ. All placentae appeared normal. Fetal alterations: all gross external, soft tissue and skeletal alterations (malformations and variations) were considered unrelated to administration of 4 dosages the test article. Fetal ossification site averages were unaffected. The liter averages per site per fetus were comparable among the 4 dosage groups and did not significantly differ.

Comments: Based on the results of this study, maternal toxicity was evident in the 450 mg/kg/day dosage group as manifest by adverse clinical observations (localized alopecia), transient body weight loss and reductions in feed consumption. A dosage level of 150 mg/kg/day may be considered the maternal NOEL for GS-4331-05. Based on a body surface area conversion factor, an equivalent dosage in humans would be 24.35 mg/kg/day. The fetal developmental NOEL was 450 mg/kg/day; an equivalent dosage in humans would be 73 mg/kg/day.

At the time of this study, in the clinic, the sponsor was using the following doses of bis-POCPMPA for a period of 4 weeks: 75, 150, 300, 600 or 900 mg/day.

4. Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of bis-POCPMPA in rats, Lot # 2454-A-2P, Gilead Sciences, Inc., Foster City, CA, January 26, 2001, (R990202)

Groups of presumed pregnant rats [Crl:CD (SD) IGR VAF/Plus; weights: 206-247 g; 25 rats/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 50 (low), 150 (mid), 450 (high) or 600 mg/kg/day (very high) once daily beginning day 7 of presumed gestation and continuing through day 20 of lactation. Blood samples were collected predosage, 15 and 40 min, and 1, 2, 4, 6, 8, 12 and 24 hr postdose. At approximately 90 days of age, the F1 generation rats were assigned to cohabitation. Results-F0 generation rats: no drug related death occurred. Clinical observations of red perioral substance and excess salivation occurred in an increased or statistically significantly ($p<0.05$ to $P<0.01$) increased numbers of rats (mid, high or very high). Dehydration ,and emaciation occurred in increased or statistically significantly increased numbers of rats (2 dams in high or 3 dams in very high). Necropsy observations: terminal body weights, paired kidney weights and the ratios of the kidney weights to the terminal body weights were comparable among the groups. Body weights: gains for the entire gestation dosage period were statistically significantly reduced in rats (mid, high or very high). During the lactation period, maternal weight gains tended to be increased over or comparable to the control group values (mid, high or very high). Absolute (g/kg) and relative (g/kg/day) feed consumption values during gestation were statistically significantly reduced in rats (high and very high). for the entire gestation dosage period. Peripartum/postpartum pup mortality and still births were increased (high and very high) and there was a concomitant significant reduction in the percentage of liveborn pups (very high). The percentage of pups found dead or presumed cannibalized were increased or statistically significantly increased in high or very high dosage groups. Pup weights were statistically significantly reduced in the high and very high dosage groups. Increased or statistically significantly increased numbers of F1 generation litters has pups that were cold to touch, pale and had umbilical hernias (high and very high). Weights of kidneys were statistically significantly reduced (high and very high) reflecting the statistically significantly reduced average body weights in these pups. F1 generation rats: one and two male rats in the high and very high groups, respectively, were fund dead. One male rat (very high) was sacrificed moribund. Two females in each of the high and very high groups were found dead. One female rat (high) was sacrificed moribund. Necropsy observations: of empty stomach and small and large intestines distended with gas, dark red area on the glandular mucosal surface of the stomach and tan spleen were considered to be associated with dead or moribund sacrificed male and female rats. Dosage dependently reduced to statistically significantly reduced terminal body weights of the F1 generation male rats were observed at all dose levels. Paired

testes and epididymides weights were reduced and statistically significantly reduced (high and very high) groups. Reflecting the reductions in pup body weights during the preweaning period, body weights and body weight gain of the F1 generation rats in all treated groups were reduced or statistically significantly reduced during the postweaning period. Absolute feed consumption values during the postweaning period for the F1 generation males were reduced to statistically significantly reduced in all treated groups. Absolute feed consumption values during the postweaning period for the F1 females were statically significantly reduced (very high). The average day on which preputial separation was evident in male rats was statistically significantly increased and was dose dependent for the male rats whose dams were administered high or very high doses. The time to reach vaginal patency in female rats was prolonged in dams (high or very high). The passive avoidance paradigm, male and female rats (high) had first trial latencies that were statistically significantly increased. There were no biologically important differences in the values for learning, short-term retention, long-term retention or response inhibition in the male or female rats, as evaluated by performance in a watermaze swim task. Mating performance of the F1 male and female rats was unaffected by administration of drug to the F0 dams.

Comments: On the basis of these data, the maternal NOEL for oral bis-POCPMPA was 50 mg/kg/day. The developmental NOEL was 150 mg/kg/day. The NOEL for general toxicity in the F1 generation was 50 mg/kg/day. The F1 generation male and female NOEL for behavior, reproductive and developmental toxicity was 50 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 8.11, 24.35, 8.11 and 8.11 for the maternal, developmental, general toxicity for F1 and behavior toxicity, respectively.

5. Oral (stomach tube) developmental toxicity study of bis-POCPMPA in Rabbits, Lot # 2454-A-2P, Gilead Sciences, Inc., Foster City, CA, June 7, 1999, (98-TOX-4331-005)

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: one doe (high) aborted and was sacrificed on gestation day 20. This abortion was considered drug-related because it occurred in the highest dose group. All other does survived until scheduled sacrifice. The number of does with scant feces (high) was significantly increased. A red

substance in the cage pan was observed for two rabbits (high) on gestation days 19 and 20. No necropsy findings were considered drug-related. Maternal body weight gains were significantly ($p<0.01$) reduced (69%; high) for the entire dosage period. During the postdosage period (gestation days 19-29), body weight gains were significantly increased (high). Significant body weight loss or reduced body weight gains occurred (high) on gestation days 8-19. Maternal absolute (g/day) and relative (g/kg/day) feed consumption values were significantly ($p<0.01$) reduced (high) for the entire dosage period. No Caesarean-sectioning or litter parameters were affected by the treatment. There were no dosage-dependent or biologically important differences in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations.

Comments: On the basis of these data, the NOEL for the female rabbits was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 31.94 mg/kg/day. The developmental NOEL was 300 mg/kg/day; a human equivalent dose would be 95.84 mg/kg/day.

Genetic toxicology-Tenofovir DF

1. Mutagenicity test with bis-POCPMPA in Salmonella - Escherichia coli/mammalian microsome reverse mutation assay, Lot # 1156-27-13, Corning Hazelton Wisconsin, Inc., Vienna, VA, February 7, 1997, (96-TOX-4331-05)*

Bis-POCPMPA was evaluated for mutagenic activity in the Bacterial Reverse Mutation Assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, and Escherichia coli strain WP2uvrA. The assay 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. The test compound was studied at concentrations ranging from 100 to 5000 µg/plate. Results: bis-POCPMPA caused positive (1.2 to 3.2 fold) increases in the mean number of revertants per plate with tester strain TA 1535 only in the absence of S9 mix. No other positive increases in the mean number of revertants per plate were noted with any of the remaining tester strain/activation condition combinations. Conclusions: under the conditions of this study, bis-POCPMPA was found to be mutagenic.

2. Mutagenicity test with bis-POCPMPA in the L5178Y TK+/- mouse lymphoma forward mutation assay, Lot # 4331-05-XA-1, Covance, Vienna, VA, July 30, 1998, (97-TOX-4331-07)

This study was designed to evaluate the potential of bis-POCPMPA to induce mutations at the thymidine kinase (TK) locus in cultured L5178Y cells in the presence and absence of an exogenous

metabolic activation system (S9). The S9 homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™-1250 at a dose level of 500 mg/kg. Bis-POCPMPA was evaluated at concentrations of 0, 12.5, 25, 50, 62.5, 75, 100, 125 or 150 µg/ml with and without S9. Results: dose related increases in mutant frequency were seen in both the presence and absence of the S9 system. The increases ranged from a 3.1-fold to 16.4-fold (without S9 system) and 5-fold to 8.6-fold (with S9 system) above the background mutant frequency. Conclusions: under the conditions of the study, bis-POCPMPA was found to be positive in inducing gene mutations.

3. In vivo mouse micronucleus assay of bis-POCPMPA, Lot # TX4331-05-XA-1, Covance Laboratories, Inc., Vienna, VA, November 4, 1998, (97-TOX-4331-008).

Groups of male Crl:CD-1 mice (6 animal/dose level/harvest timepoint) were administered a single dose bis-POCPMPA via oral gavage at dose levels of 0 (vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg (high) to evaluate the test article for in vivo clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in the bone marrow. The dose levels were selected based upon the results of a dose-range finding study of bis-POCPMPA. Results: bis-POCPMPA induced no signs of clinical toxicity in any of the treated animals and was not cytotoxic to the bone marrow (ie, no statistically significant decrease in the PCE:NCE ratio). The test compound did not induce a statistically significant increase in micronuclei in bone marrow PCEs. Conclusions: bis-POCPMPA is considered negative in the mouse bone marrow micronucleus test under the conditions of exposure in this assay.

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4. Mutagenicity test with PMPA in *Salmonella*/Mammalian microsome reverse mutation assay, Lot # KH01603, 1278-B-1 and 4331-05-XA-1 (PMPA prodrug), Covance Laboratories, Vienna, VA, March 6, 1998, (97-TOX-1278-003)

PMPA was evaluated for mutagenic activity in the Bacterial Reverse Mutation Assay using Salmonella typhimurium strain TA1535. The assay was conducted in the presence of a metabolic activation system using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. The test compound was studied at concentrations ranging from 100 to 5000 µg/plate. Results: PMPA did not cause positive increases in the mean number of revertants per plate with tester strain TA 1535 in the presence of S9 mix.

Conclusions: under the conditions of this study, PMPA was not found to be mutagenic.

12. Mutagenicity test with PMPA in *Salmonella - Escherichia coli*/Mammalian microsome reverse mutation assay, Lot # 1016-56-26, Corning Hazelton Wisconsin, Inc., Vienna, VA, June 10, 1996, (95-TOX-1278-006/CHV 17444-0-409)*

PMPA was evaluated for mutagenic activity in the Bacterial Reverse Mutation Assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, and Escherichia coli strain WP2uvrA. The assay 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. The test compound was studied at concentrations ranging from 92.2 to 5000 µg/plate. Results: PMPA caused positive (6.2-6.8 fold) increases in the mean number of revertants per plate with tester strain TA 1535 only in the presence of S9 mix. No other positive increases in the mean number of revertants per plate were noted with any of the remaining tester strain/activation condition combinations. Conclusions: under the conditions of this study, PMPA was found to be mutagenic.

3. Mutagenicity test with PMPA in the L5178Y TK +/- mouse lymphoma forward mutation assay, Lot # 1016-56-26, Corning Hazelton Wisconsin, Inc., Vienna, VA, May 29, 1996, (95-TOX-1278-007/CHV 17444-0-431)*

The potential of PMPA to induce mammalian cell mutations was studied in the L5178Y TK+/- mouse lymphoma cell assay. The test was conducted both with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254-treated rats. Six treatments from 156 to 5000 µg/ml were studied. Results: in the presence of S9 metabolic activation, the 5000 µg/ml treatment induced approximately 2.5-fold increase in the mutant frequency that exceeded the minimum criterion. Moderate to no cytotoxicity was observed at the test article concentrations studied. In the absence of metabolic activation, there was no cytotoxicity and evidence of mutation. Conclusions: under the conditions of this study, PMPA was found to be mutagenic.

PHARMACOKINETICS

Tenofovir DF

1. In vitro stability of bis-POCPMPA in biological fluids (97-VIT-1278-001)

In an in vitro experiment, bis-POCPMPA was incubated with plasma (rat, dog and human) and microsomes from rat, dog and human

tissues (liver and intestine) at 37°C for 60 min to evaluate the stability of the compound. Concentrations of bis-POCPMPA were determined using a chiral HPLC assay. Results: bis-POCPMPA was hydrolyzed rapidly (t½ = 5 min) in human plasma, intestinal homogenate and liver homogenate, and dog liver homogenate (t½ = 5 min). Hydrolysis in dog plasma (t½ = 20.5 min) and dog intestinal homogenate (t½ = 52.6 min) was considerably slower. In all cases, the primary metabolite formed was the monoester.

2. In vivo mouse micronucleus assay of bis-POCPMPA-PK portion (97-TOX-4331-008-PK).

Groups of male Crl:CD-1 mice (6 animal/dose level/harvest timepoint) were administered a single dose bis-POCPMPA via oral gavage at dose levels of 0 (vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg (high). Plasma tenofovir levels were determined using a validated HPLC method. Results: are shown in Table 1.

Table 1

Mean PMPA levels (µg/ml) following a single dose bis-POC PMPA (500, 1000 or 2000 mg/kg) administration to male mice

Time (hr)	Dose (mg/kg)		
	500	1000	2000
1	3.72	8.12	28.1
24	0.68	1.2	2.05
48	0.05	0.15	0.92

3. Pharmacokinetics and oral bioavailability of PMPA prodrugs GS4326 and GS4331 in Sprague-Dawley rats (96-DDM-1278-007)

The oral bioavailability of two prodrugs of PMPA (GS1278) were determined in Sprague-Dawley rats. The two prodrugs were bis-(ethoxycarbonyloxymethyl)PMPA or bis-(Et COM)PMPA, GS4326 and bis-POCPMPA, GS4331. 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 2. No intact prodrug or monoester were observed in rat plasma following oral administration of either prodrug.