CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-356

PHARMACOLOGY REVIEW

PHARMACOLOGIST'S REVIEW

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SPONSOR: Gilead Sciences, Inc.
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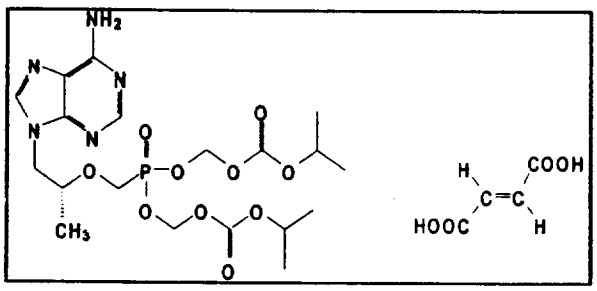

INFORMATION TO SPONSOR: No

DRUG: Tenofovir disoproxil fumarate or Tenofovir DF
Proposed brand name: Viread
Other name: Bis-POCPMPA fumarate or PMPA Prodrug
Chemical Name: 9-[2-(R)-[[bis[[(isopropoxycarbonyl)oxy]methoxy]-phosphinoyl]methoxy]propyl]adenine fumarate (1:1) (IUPAC)
Other Name: Bis(1-methylethyl)(R)-5-[[2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8-teraoxa-5 phosphanonanedioate,5-oxide,(E)-2-butenedioate (1:1)
CAS Reg. No.: 202138-50-9 (fumarate salt)
Research Compound No.: GS-4331-05
Empirical Formula: $C_{23}H_{34}O_{14}N_5P$
Molecular Weight: 635.52
Melting Point: 112-119°C
Physical Appearance: White to off-white crystalline powder
Solubility: 14-43 mg/ml in aqueous buffers between pH 1-6.5
pKa: 3.75

ROUTE OF ADMINISTRATION: Oral tablets.

FORMULATIONS: Bis-POCPMPA prodrug tablets are supplied as white to off-white, round, [redacted] tablets. Each tablet contains 75 mg of active drug. The inactive ingredients included lactose monohydrate [redacted] pregelatinized

starch [redacted] croscarmellose sodium [redacted]
magnesium stearate [redacted]

INDICATION: Treatment of human immunodeficiency virus (HIV) infection

RELATED INDs: [redacted]

INTRODUCTION AND DRUG HISTORY:

Bis-POCPMPA fumarate or PMPA prodrug (GS-4331-05) is an alkoxycarbonyloxy ester prodrug of PMPA (GS-1278). The prodrug is orally bioavailable in animals and is rapidly converted in vivo to PMPA [redacted]; and subsequently to PMPA-diphosphate (PMPApp, the active metabolite of PMPA). In the in vitro metabolism studies, the monoester was identified as the primary metabolite of PMPA prodrug. PMPA is a nucleotide analog belonging to the class of acyclic phosphonomethylether nucleoside and structurally closely related to 9-[2-(phosphonomethoxy)-ethyl] alanine (PMEA, the subject of IND [redacted] differing by only one additional methyl group). In in vitro experiments, similar to PMEA, PMPA exhibits antiviral activity against retroviruses and hepadnaviruses. The cellular enzyme responsible for PMPA metabolism to PMPApp is adenylate kinase. PMPApp competitively inhibits both RNA- and DNA-directed reverse transcriptase DNA polymerase activities. PMPApp competes with dATP for incorporation into nascent DNA and causes premature chain termination.

The nonclinical toxicity of tenofovir DF has been studied in mice, rats, dogs, rabbits and monkeys. The target organs of toxicity associated with tenofovir DF were the gastrointestinal tract (primarily in rodents), the renal tubular epithelium of all species, and bone of all species except rabbits. The dose-limiting toxicity in rodents was gastrointestinal. Histopathological alterations typically included inflammation of the stomach and intestines, epithelial cytomegaly in the duodenum and jejunum and villous atrophy of the ileum. The most severe GI effects occurred acutely at the high doses, generally greater than 300 mg/kg/day. The mechanism of this toxicity is apparently related to the high local tenofovir DF concentrations to which GI epithelium was exposed. The dose-limiting toxicity associated with tenofovir DF administration in dogs (45 mg/kg/day) and monkeys (600 mg/kg/day) was nephrotoxicity. Microscopic alterations in dogs treated for 42 weeks at doses >10 mg/kg/day included renal tubular epithelial karyomegaly and individual cell necrosis. The dog is the most sensitive species in relation to

kidney toxicity.

Bone mineral loss resulting in pathologic osteomalacia was observed in juvenile rhesus monkeys following treatment with high doses (30 mg/kg) of tenofovir DF administered subcutaneously in SIV efficacy studies; thus, specialized parameters to evaluate potential bone effects were added to 13/42 week rat and dog studies. Reduced bone mineral density and contents were seen in distal femoral metaphyses and mid-femoral diaphyses after 13 and 42 weeks of orally administered tenofovir DF in rats and dogs. There was increased urinary excretion of calcium, deoxypyridinoline (rats) and N-telopeptide (dogs), and a rise in the serum PTH and osteocalcin (rats), and bone-ALP (dogs). The NOELs for bone effects in rats was 100 mg/kg/day and in dogs was 10 mg/kg/day.

Tenofovir DF has been shown to decrease phosphate absorption from the intestinal tract in rats and monkeys. Decreased serum phosphate concentrations have also been reported in some patients in long-term clinical studies.

SUMMARY:

Single dose toxicology-Tenofovir DF:

Groups of male and female Sprague-Dawley rats were orally gavaged with single doses of bis-POCPMPA at dose levels of 0, 160, 500 or 1500 mg/kg. There were no deaths or drug related clinical signs in the study. A dose level of 1500 mg/kg may be considered the NOAEL. Based on the body surface area factor, an equivalent dose in humans would be 243.5 mg/kg (14.6 g/day for a 60 kg person). Groups of male and female beagle dogs were orally gavaged with single doses of BIS-POCPMPA at dose levels of 0, 30, 90 or 270 mg/kg. There were no deaths during the study. Drug-related lesions were observed in the kidneys of both male and female animals at the mid and high doses. A dose level of 30 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 16.2 mg/kg (973 mg/day for a 60 kg person).

Multiple dose oral toxicology-Tenofovir DF:

Groups of male and female ICR CD-1 mice were orally gavaged with bis-POCPMPA at dose levels of 0, 100, 300 or 1000 mg/kg/day for 13 consecutive days. The findings of this study indicate that the liver, kidney and bone marrow were the target organs of toxicity. In this study, a NOAEL could not be determined; it should be considered to be lower than 100 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be less

than 8.11 mg/kg (486 mg/day for a 60 kg person). Groups of male and female albino mice were orally gavaged with bis-POCPMPA suspension at dose levels of 0, 100, 300, 600 or 1000 mg/kg/day for a period of 13 consecutive weeks. In the kidneys, a minimal tubular karyomegaly characterized by a slight enlargement of some nuclei was noted in all treated groups: low (4/15 females), mid (9/15 females and 6/15 males) and high (14/15 females and 8/15 males). In the duodenum, epithelial hypertrophy in the mucosa featured enlarged epithelial cell with increased amounts of cytoplasm and was present in mid (3/15 males and 6/15 females) and high (13/15 males and 14/15 females) dose groups animals only. A NOAEL for oral bis-POCPMPA in mice could not be identified; it should be considered less than 100 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 8.11 mg/kg/day or 486.6 mg/day.

In a 5-day study in male Sprague-Dawley rats, administration of bis-POCPMPA at dose levels of 0, 25, 100 or 400 mg/kg/day resulted in reduced terminal body weights and body weight gains at the high dose only. The NOEL for oral bis-POCPMPA in male rats was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 16.23 mg/kg/day. In a 4-week oral toxicity study of bis-POCPMPA in male and female Sprague-Dawley rats, in the administrations of bis-POCPMPA at dose levels of 0, 20, 100 or 500 mg/kg/day, a target organ could not be identified in the study. The absolute and relative kidney weights were decreased significantly in males (high). Slight (less than 2-fold) increases in ALT values occurred in males and females (high) and slight decreases were seen in total protein, albumin and sodium values in females (high). The NOEL for oral bis-POCPMPA in male rats was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 16.23 mg/kg/day. In a 13-week oral gavage toxicity study (with a 13-week recovery period) of bis-POCPMPA in the albino rat, groups of male and female Sprague-Dawley rats were orally gavaged with bis-POCPMPA at dose levels of 0, 30, 100, 300 or 1000 for 13 weeks. Histological findings: related to the treatment were seen in the kidneys and gastrointestinal tract. 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). The NOEL for oral bis-POCPMPA in male and 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 16.23 mg/kg/day. In a 42-week oral gavage toxicity study (with a 13-week recovery period) of bis-POCPMPA in the albino rat, groups of male and female Sprague-Dawley rats were orally gavaged with bis-POCPMPA at dose levels of 0, 30,

100, 300 or 1000 mg/kg/day once daily for 42 consecutive weeks followed by a 13-week recovery period. Histological findings: related to the treatment were seen in the kidneys and gastrointestinal tract. 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 times 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 increased 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. 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 a 5-day study in male beagle dogs, the administration of bis-POCPMPA at dose levels of 0, 9, 45 or 180 mg/kg/day resulted in bone marrow, GI tract, kidney and lymphoid tissue toxicities at the high dose. Mid dose: drug-related findings included 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. 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. In a 4-week oral toxicity study of bis-POCPMPA in male and female beagle dogs, the administrations of bis-POCPMPA at dose levels of 0, 3, 10 or 30 mg/kg/day resulted in kidney

toxicity at the mid and high dose levels. The NOEL for oral bis-POCPMPA in 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. In a four week oral gavage toxicity study of bis-POCPMPA in the beagle dog, groups of male beagle dogs were orally gavaged with bis-POCPMPA at dose levels of 0 (bid), 15 (bid), 30 (qd), 60 (q2d) or 210 mg/kg/dose (q7d) over a 4-week period. 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 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). Greater toxicity including deaths were observed when drug was administered as a single dose of 210 mg/kg. A NOEL for oral bis-POCPMPA in dogs could not be identified; it should be considered less than 15 mg/kg/day bid. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 486 mg bid. In a 13-week oral gavage toxicity study of bis-POCPMPA in the beagle dog, groups of male and female beagle dogs were orally gavaged with bis-POCPMPA at dose levels of 0, 3, 10 or 30 mg/kg/day for 13 weeks. Histopathologic findings: drug-related changes were seen in the kidneys of male and female dogs (mid and high). 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 97.2 mg/kg/day. In a 42-week oral gavage toxicity study of bis-POCPMPA in the beagle dog, groups of male and female beagle dogs were orally gavaged with bis-POCPMPA at dose levels of 0, 3, 10 or 30 mg/kg/day once daily for 42 weeks. Gross and histopathologic findings: drug-related gross lesions were observed in the kidneys (mid and high). 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. 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).

Single and multiple dose toxicology-Tenofovir

In a single dose intravenous toxicity study of PMPA in Sprague-Dawley rats, groups of male rats received single iv bolus injections of PMPA. 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. In a fourteen day intravenous infusion toxicity and toxicokinetics study of PMPA in Sprague-Dawley rats, groups of male and female rats received iv infusions (over 60 min period) of PMPA at dose levels of 0, 5, 25 or 100 mg/kg/day once daily for 14 days. 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. In a fourteen day intravenous infusion toxicity study of PMPA in Cynomolgus monkeys, groups of male and female monkeys received one hr iv infusions of PMPA at dose levels of 0, 3, 10 or 25 mg/kg/day once daily for 14 consecutive days. 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 to Tenofovir and Tenofovir DF

In 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, groups of male rats 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. 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. In 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, groups of male rats 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 rats treated with iv/po tenofovir or tenofovir DF by oral gavage were changed by supplementation with oral phosphate immediately following the final dose. 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. In a 28-day study to evaluate the effects of bis-POCPMPA on bone following daily administration by gavage in the Sprague-Dawley rat, groups of male rats were orally gavaged with bis-POCPMPA at dose levels of 0, 40 or 400 mg/kg/day once daily for 28 days to evaluate the effects of tenofovir on biochemical markers of bone turnover. 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: 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 by osteoclast surface. Bone formation and mineralization remained unaffected by the treatment. 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.

Summary of PMPA in SIV-infected and uninfected rhesus monkey, the summary reports (Submission # 9) indicated that bone lesions were seen in 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 treated throughout life. 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 [redacted] 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 undergoing long term PMPA treatment at 30 mg/kg/day ranged from [redacted] 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. Summary of PMPA toxicity data in rhesus monkey, Univ of California; 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 reduce clearance of PMPA and induced a Fanconi-like syndrome (proximal renal tubular disorder), with glucosuria and hypophosphatemia. The 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 are safe. Evaluation of radiographs from control and PMPA treated juvenile rhesus monkeys; 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. The daily administration of PMPA at a dosage of 10 mg/kg by sc injection resulted in exposure values ranging from 10 to 18 µg*hr/ml. A 56-day study of tenofovir DF administered orally and of PMPA administered by sc injection to rhesus monkeys; groups of male and female monkeys received a dose of the control article (50 mM citric acid solution, controls) or tenofovir DF orally at dose levels of 30, 250 or 600 mg/kg/day or PMPA via sc injection (30 mg/kg/day) once daily for 56 consecutive days. Based on the results of the study, kidney, testes and thymic lymphoid tissue 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. Effect of PMPA treatment on cortical bone strength in rhesus monkeys, thesis, 2000; chronic treatment of PMPA (30 mg/kg/day) can result in a mineralization defect 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. PMPA summary report: Human osteoblast calcium deposition in vitro; 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. PMPA did not alter cellular calcium deposition, nor result in HOLC cytotoxicity.

Special toxicology-Tenofovir DF

In a primary eye irritation study of bis-POCPMPA in rabbits, New Zealand White rabbits received a single application 0.076 g (0.1 ml) of bis-POCPMPA in the right eyes. Bis-POCPMPA was considered to be a very severe irritant to the ocular tissue of the rabbit. In a primary skin irritation study of bis-POCPMPA in rabbits, 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 considered to be a slight irritant to the rabbit skin. In a dermal sensitization study of bis-POCPMPA in guinea pigs, male and female guinea pigs were topically treated with 75% bis-POCPMPA in polyethylene glycol 400 once per week for three consecutive weeks to evaluate the dermal sensitization potential of the test compound. The test article was not considered to be a contact sensitizer in guinea pigs. In a guinea pig ileum contractile response test, 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. These results suggest that high concentration of bis-POC PMPA have direct inhibitory effects on smooth muscle contractility.

Reproductive toxicology-Tenofovir DF

In an oral (gavage) fertility and general reproduction toxicity of bis-POCPMPA in rats, groups of male rats were orally gavaged with bis-POCPMPA at dose levels of 0, 100, 300 or 1000 mg/kg/day once daily for 9 days. Because of the unacceptable mortality and moribundity (high), the study was terminated. In an oral (gavage) fertility and general reproduction toxicity study of bis-POCPMPA in Sprague-Dawley rats, groups of male and female rats were orally gavaged with bis-POCPMPA at dose levels of 0, 100, 300 or 600 mg/kg/day 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. Microscopic observations included 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). 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. In an oral (gavage) developmental toxicity study of GS-4331-05 in rats, groups of presumed pregnant female Sprague-Dawley rats were administered GS-4331-05 via oral gavage at dose levels of 0, 50, 150 or 450 mg/kg/day on days 7-17 of presumed gestation. 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. In an oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of bis-POCPMPA in rats, groups of presumed pregnant rats were orally gavaged with bis-POCPMPA at dose levels of 0, 50, 150, 450 or 600 mg/kg/day once daily beginning day 7 of presumed gestation and continuing through day 20 of lactation. 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. In an oral (stomach tube) developmental toxicity study of bis-POCPMPA in Rabbits, groups of female New Zealand White rabbits were orally administered (stomach tube) bis-POCPMPA at dose levels of 0, 30, 100 or 300 mg/kg/day once daily on days 6-18 of presumed gestation. 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

Mutagenicity test with bis-POCPMPA in Salmonella - Escherichia coli/mammalian microsome reverse mutation assay; 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 [redacted] induced rats. The test compound was studied at concentrations ranging from 100 to 5000 µg/plate. Under the conditions of this study, bis-POCPMPA was found to be mutagenic. Mutagenicity test with bis-POCPMPA in the L5178Y TK+/- mouse lymphoma forward mutation assay; 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) was evaluated at concentrations of 0, 12.5, 25, 50, 62.5, 75, 100, 125 or 150 µg/ml with and without S9. Bis-POCPMPA was found to be positive in inducing gene mutations. In vivo mouse micronucleus assay of bis-POCPMPA; groups of males were administered a single dose of bis-POCPMPA

via oral gavage at dose levels of 0, 500, 1000 or 2000 mg/kg to evaluate the test article for in vivo clastogenic activity and/or disruption of the [redacted] apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in the bone marrow. Bis-POCPMPA is considered negative in the mouse bone marrow micronucleus test under the conditions of exposure in this assay.

Genetic toxicology Tenofovir

Mutagenicity test with PMPA in *Salmonella*/Mammalian microsome reverse mutation assay; PMPA was evaluated for mutagenic activity in the Bacterial Reverse Mutation Assay using Salmonella typhimurium strain TA1535. The test compound was studied at concentrations ranging from 100 to 5000 µg/plate. PMPA was not found to be mutagenic. Mutagenicity test with PMPA in *Salmonella - Escherichia coli*/Mammalian microsome reverse mutation assay; 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 test compound was studied at concentrations ranging from 92.2 to 5000 µg/plate. PMPA was found to be mutagenic. Mutagenicity test with PMPA in the L5178Y TK +/- mouse lymphoma forward mutation assay, six treatments from 156 to 5000 µg/ml were studied. PMPA was found to be mutagenic.

PHARMACOKINETICS:

The absorption, distribution, metabolism and excretion of tenofovir and tenofovir DF have been investigated in mice, rats, dogs and monkeys. Following oral administration of tenofovir DF in these species, maximum tenofovir plasma concentrations were reached at 0.25 to 1.5 hr and declined in a biphasic manner. The observed terminal half-life values were approximately 7, 9 and 60 hr in rats, monkeys and dogs, respectively. Due to the long terminal half-life in dogs, a substantial degree of accumulation was observed upon daily repeat dosing in this species. The oral bioavailability of tenofovir DF was greater in dogs and monkeys (30-40%) and least in rodents (10-20%). The prodrug moiety was efficiently cleaved in all species tested.

No circulating metabolites of tenofovir, other than the monoester observed at early time points in rats and dogs, were detected. This was consistent with the lack of metabolism of tenofovir in intestinal and liver preparations. A small but statistically significant degree of CYT P450 induction (CYP 1A and 2B) was observed in livers from rats given daily 400 mg/kg/day doses of tenofovir DF for 28 days. Extensive tissue distribution, suggested by the plasma pharmacokinetics of tenofovir, was confirmed in studies with ^{14}C-labeled tenofovir in dogs. Major site of tissue uptake included the liver and kidney.

Tenofovir was excreted unchanged in the urine of all animal species tested and renal excretion was identified as the primary route of elimination. Elimination of tenofovir in milk was observed in both lactating rats and monkeys with concentrations that were significantly lower than those observed in plasma (14-24% in rats and 2-4% in monkeys). The tenofovir $AUC_{(0-\infty)}$ in monkey milk was approximately 20% of the observed AUC value in plasma.

Studies evaluating pharmacokinetics of the test compound: the stability of bis-POCPMPA was examined in rat, dog and human plasma and in homogenates of dog and human intestine and liver. Bis-POCPMPA was hydrolyzed rapidly ($t_{1/2}$ = 5 min) in human plasma, intestinal homogenate and liver homogenate and dog liver homogenate ($t_{1/2}$ = 5 min). Hydrolysis in dog plasma ($t_{1/2}$ = 20.5 min) and dog intestinal homogenate ($t_{1/2}$ = 52.6 min) was considerably slower. In all cases, the primary metabolite formed was the monoester. In mouse, concentrations of tenofovir were determined in plasma samples following oral gavage of tenofovir DF in albino mice at doses of 100, 300 or 1000 mg/kg. Tenofovir DF was rapidly absorbed and converted to tenofovir with Tmax values that ranged from 0.083 to 0.5 hr and Cmax values of 3.38, 5.89 and 35.1 µg/ml for the 100, 300 and 1000 mg/kg dose groups, respectively. In repeat dose oral studies in mice for 13 weeks at doses of 100, 300 or 1000 mg/kg/day, the Cmax values were 8.82, 17.9 and 33.8 µg/ml and AUC values were 14.6, 35.8 and 61.7 µg*hr/ml. In male rats, concentration of PMPA in plasma following oral administration of bis-POCPMPA reached a maximum of 0.22 µg/ml at 0.75 hr postdose. Thereafter, the concentration of bis-POCPMPA declined in a bi-exponential manner with a terminal half-life of 6.8 hr. The mean oral bioavailability of PMPA from bis-POCPMPA was 13.1%. Rats were administered 10 mg-equiv. of tenofovir/kg (containing 25 µCi ^{14}C-tenofovir DF/kg. Gastrointestinal contents and tissues were harvested 1 hr postdose. Stomach, intestinal tissues and contents were processed and concentrations of total radioactivity were determined by direct scintillation counting. Approximately 65% of the total dose was recovered in gastrointestinal contents 1 hr post dose. Intact tenofovir DF (prodrug) was the major radioactive species (>85%) detected in the stomach and its contents. The biliary excretion of tenofovir DF in rats over 24 hr post dose following the oral administration was negligible. At 1 hr post dose, the majority of the dose remained in the intestinal tract, particularly the ileum. Intact tenofovir DF was the major radioactive species detected in the stomach and its contents; whereas, tenofovir was the major metabolite detected in the small intestinal tissues and contents. Pharmacokinetics of bis-POCPMPA in a 28-day toxicity study in albino rats; groups of male and

female albino rats were orally gavaged with bis-POCPMPA at dose levels of 0, 20, 100 or 500 mg/kg/day once daily for 4 consecutive weeks. The pharmacokinetics of tenofovir in rat appeared to be dose-dependent based on Cmax. Following oral administration of tenofovir on day 1, Cmax deviated from dose proportionality over the range of 20 to 500 mg/kg/day, indicating decreased absorption at the higher dose. In male beagle dogs, following oral administration of bis-POCPMPA, concentrations of PMPA reached a maximum of 2.5 µg/ml at 0.7 hr postdose. Thereafter, the concentrations of bis-POCPMPA declined in a bi-exponential manner with a terminal half-life of 16.2 hr. Intact bis-POCPMPA was not observed in plasma following the oral administration. The bioavailability of PMPA from bis-POCPMPA was 30.1%. Urinary recovery of PMPA following the oral administration of bis-POCPMPA accounted for 21.1% of the administered dose in 48 hr. Monkeys: single doses of both intravenous PMPA (10 mg/kg) and oral (gavage) bis-POCPMPA (10 mg-equiv. of PMPA/kg) resulted in concentrations of PMPA that declined in a bi-exponential manner. Clearance (0.71 L/hr/kg) was higher than GFR in the animal. The bioavailability was 4.9%.

Distribution: The tissue distribution and recovery of radioactivity were examined in beagle dogs following oral administration of ^{14}C-radiolabeled tenofovir DF (10 mg/kg; 25 µCi/kg). At one hr post dose, radioactivity was detected in all tissues except brain. The majority of radioactivity was present in the contents of GI tract, jejunum and liver (>66%). Concentrations were highest in bile, kidney, liver and jejunum. By six hr post dose, the highest concentrations of radioactivity were present in kidney, liver and the intestinal contents. Protein binding: of PMPA was determined in human plasma and serum using ultracentrifugal filtration; the percent unbound for PMPA was 99.1% in human plasma and 92.6% in human serum.

Metabolism: The in vitro metabolism of tenofovir DF was studied in rats and dogs. Tenofovir DF was rapidly converted to the monoester in all systems. No metabolites were detected in rat microsomal preparations.

Excretions: The routes of excretion were evaluated in beagle dogs following oral administration of radiolabeled tenofovir DF (10 mg/kg). Total recovery of the dose at 24 hr was >90% (24-44% in feces, 4-24% in GI contents, 24% in urine, 15-20% in tissues and 1-4% in cage wash). The amount of dose recovered in urine and the cage wash (25-28%) combined with the amount remaining in tissues (15-20%) were consistent with an oral bioavailability of 31-44%. The data are consistent with renal elimination as the primary path of excretion of tenofovir following absorption of tenofovir DF and metabolism of tenofovir.

Tenofovir

Studies evaluating ADME of the test compound were conducted in rats, dogs and monkeys. PMPA levels in plasma declined bi-exponentially following iv administration, with half-lives ranging from 1.9 hr in monkeys to 7 hr in rats. Absolute oral bioavailability of PMPA was low, ranging from 3-5% in monkeys to 18% in dogs. High steady state volume of distribution for PMPA suggested distribution beyond the extracellular fluid. Total plasma clearance of PMPA exceeded the glomerular filtration rate, suggesting active renal tubular secretion. The pharmacokinetics of iv PMPA in tested species were dose-dependent. The dose-dependent pharmacokinetics appeared to be due to saturation of the tubular secretion pathway as demonstrated by a significant longer half-life of PMPA elimination and slower clearance at higher doses. Repeated administration of PMPA significantly altered the pharmacokinetics in Cynomolgus monkeys (75 mg/kg/day, high), which resulted in an increased Cmax and AUC and decreased clearance, consistent with nephrotoxicity observed at this dose. In studies of PMPA in rats and beagle dogs, drug was primarily excreted in urine as unchanged drug. The rate of urinary excretion of iv PMPA dose levels appeared to be dose-dependent in both species. For example, in the dog urinary recovery of PMPA in 48 hr decreased from 84% after a 1 mg/kg dose to 75% after a 10 mg/kg dose. Intravenous administration of PMPA to pregnant Cynomolgus monkeys resulted in a fetal/maternal concentration of 17%, suggesting significant transfer of PMPA across the placenta. Intravenous administration of PMPA to newborn monkeys revealed a 5-fold lower clearance of PMPA compared to adult monkeys. In in vitro metabolic studies, PMPA was stable in human plasma, homogenates of human liver and intestine, and rat liver microsomes.

SAFETY PHARMACOLOGY:

A pharmacological assessment of the effect of bis-POCPMPA on the renal system of the rat; groups of male Sprague Dawley rats were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0, 50 or 500 mg/kg. There were no treatment related clinical signs. There were no remarkable difference between creatinine clearance values for the control and treated groups. A dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person). A pharmacological assessment of the effect of bis-POCPMPA on gastrointestinal motility in the rat; groups of male rats were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0, 50 or 500 mg/kg to observe the effects on the passage of activated charcoal along the GI tract. The increased weight of the stomach, and content and observation of charcoal in the

stomachs (high) at all time points indicated that drug reduced the rate of gastric emptying. A dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person). A pharmacological assessment of the effect of bis-POCPMPA on the central nervous system of the rat; groups of male rats were administered a single dose of bis-POCPMPA via oral gavage at dose levels of 0, 50 or 500 mg/kg to observe for apparent neuropharmacological signs following dosing. Group mean total activity counts (high) were statistically ($p<0.05$) reduced as compared to the controls. A dose level of 50 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 8.1 mg/kg (487 mg/day for a 60 kg person). A pharmacological assessment of the effect of bis-POCPMPA on the cardiovascular system of the beagle dog; a group of male beagle dogs under isoflurane/oxygen anesthesia (3 dog) were administered a single oral gavage dose of bis-POCPMPA (30 mg/kg) to determine effects on the cardiovascular/hemodynamic system over a period of 24 hr. There were no drug related effects on clinical signs, heart rate, systemic blood pressure or electrocardiograms. A dose level of 30 mg/kg may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 16.2 mg/kg (973 mg/day for a 60 kg person).

MOLECULAR PHARMACOLOGY:

Mechanism of action of PMPA - DNA polymerase inhibition:
The putative active metabolite of PMPA is PMPApp. PMPApp competitively inhibited both RNA- and DNA- directed reverse transcriptase activities. PMPApp competed with dATP for incorporation into nascent DNA and, since it lacked a 3' hydroxyl group, caused premature chain termination. For PMPApp, the Ki value (5.2 μM) of α polymerase and the Km value (2.7 μM) are rather close. The closeness of these values suggests that the test compound may have a toxic effect on the normal human α polymerase. In vitro cytotoxicity of tenofovir in various human cell types-comparison with other NRTIs; tenofovir only weakly inhibited the proliferation of liver-derived HepG2 cells and normal skeletal muscle cells with CC_{50} values > 500 μM, ZDV, ddC, ddI, d4T and abacavir all exhibited more pronounced cytotoxicity in these two cell types than tenofovir. No substantial effects on the expansion of erythroid and myeloid hematopoietic progenitor cells were observed in the presence of tenofovir at the concentrations substantially exceeding those needed for its antiviral activity in PBMCs (CC_{50} = 85 to > 200 μM vs EC_{50} = 0.2 μM). In contrast, ZDV, ddC and d4T interfered primarily with the growth of the erythroid progenitor linage with CC_{50} values ranging from 0.03 to 5 μM. Finally, tenofovir showed less effects on

proliferation and viability of renal proximal tubule epithelial cells than cidofovir and adefovir. Tenofovir exhibited weak cytotoxic effects in all cell types tested with substantially less cytotoxicity than the majority of NRTIs currently used for the treatment of HIV infection.

CONCLUSIONS:

In the clinic, the test compound is being administered as an oral formulation (300 mg tablets). The human $AUC_{0-\infty}$ (at steady state) at the dose of 300 mg/day is 3.18 µg*hr/ml. Toxicology studies conducted in rats, dogs and monkeys revealed target organ effects in gastrointestinal tract, kidney, bone and serum phosphate concentrations. Different animals species had differing levels of sensitivity to each type of effect. The GI and kidney changes were associated directly with exposure to drug. Findings in rat and monkey studies indicated that there was a direct drug-related decrease in intestinal absorption of phosphate with potential secondary reduction in bone mineral density. Reduced serum phosphate concentrations were observed only in primates and could be restored to normal by dietary phosphate supplementation. Sytemic exposures 3 to <1 times human exposures elicited minimal alterations in target organs in the most sensitive species (appendix # 4).

Tenofovir DF can be classified as Pregnancy Category B. Tenofovir DF should be used during pregnancy only if clearly needed. The U.S. Public Health Service Centers for Disease Control and Prevention advises HIV-infected women not to breast-feed to avoid post-natal transmission of HIV to a child who may not be infected.

Long term carcinogenicity studies in the rat and mouse are in progress.

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

The issue of labelling will be carried out separately.

APPENDICES:

Ten appendices are attached. These are listed below:

1. **Toxicology, pharmacokinetics/ADME and safety pharmacology studies of tenofovir DF and tenofovir.**
2. **Synopsis of tenofovir DF and tenofovir acute animal toxicity studies.**

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

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

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

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

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

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

9. Synopsis of multiple oral dose tenofovir DF pharmacokinetics in animals

10. Synopsis of single and multiple oral dose tenofovir DF pharmacokinetics in HIV-infected patients (study no. 901)

Pritam S. Verma, Ph.D.
Reviewing Pharmacologist

Concurrences:
HFD-530/JFarrelly
HFD-530/PVerma
Disk:
HFD-530\JFarrelly

Appendix # 1

Toxicology, pharmacokinetics/ADME and safety pharmacology.

TOXICOLOGY:

Toxicity Studies Summary: The studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practice Regulations.

Single dose toxicology-Tenofovir DF

1. **An acute oral gavage toxicity study of bis-POCPMPA in the albino rat followed by 14-day observation period, Lot # 2454-A-2P, ClinTrials BioResearch LTD., Senneville, Quebec, December 7, 2000, (D990200/T4331-00021)***

2. **An acute oral gavage toxicity study of bis-POCPMPA in the beagle dog followed by 14-day observation period, Lot # 2454-A-2P, ClinTrials BioResearch LTD., Senneville, Quebec, December 7, 2000, (D990201/T4331-00023)***

Multiple dose toxicology-Tenofovir DF

1. **A 14-day repeat oral gavage toxicity study of bis-POCPMPA in ICR CD-1 mice, Lot # 2454-A-2P, Gilead Sciences, Inc., Boulder, CO, January 1, 2001, (M990203/T4331-00017.1)***

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

3. **Five day repeated dose oral toxicity study of bis-POCPMPA in Sprague-Dawley male rats, Lot # 1074-191-A, Gilead Science, Inc., Foster City, CA, February 6 1997, (96-TOX-4331-002)**

4. **A 14-day repeat oral gavage toxicity study comparing tenofovir DF and degraded tenofovir DF in Sprague-Dawley rats, Lots # GS-1616-31A-degraded tenofovir DF & GS-1616-31B-tenofovir DF, MPI Research, Inc. Mattawan, MI, February 23, 2001, (T433-00024/R2000081)***

5. **Four week oral gavage toxicity study of bis-POCPMPA in albino rats, Lot # 1156-27-13, Clinical Trials Bioresearch, Sennebille, Quebec, February 25, 1997, (96-TOX-4331-003)***

6. **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 albino rat, Lot # 4331-05-XA, Clinical Trials Bioresearch, Sennebille, Quebec, Canada**

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)*

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)*

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)*

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

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

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

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)

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

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

Special toxicology-Tenofovir DF

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

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

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

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

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)

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)*
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)*

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)*

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)*

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)*

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)*

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)*

Genetic toxicology-Tenofovir

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)

5. 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)*

6. 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)*

PHARMACOKINETICS

Tenofovir DF

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Tenofovir

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

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

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

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

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

26. Single dose iv pharmacokinetics of tenofovir in rats (R2000075)

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

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

SAFETY PHARMACOLOGY:

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

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

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

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

MOLECULAR PHARMACOLOGY:

The following summary was provided from the published scientific literature.

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

2. **Mechanism of action of PMPA - DNA polymerase inhibition**

Single dose toxicology-Tenofovir DF

1. An acute oral gavage toxicity study of bis-POCPMPA in the albino rat followed by 14-day observation period, Lot # 2454-A-2P, ClinTrials BioResearch LTD., Senneville, Quebec, December 7, 2000, (D990200/T4331-00021)

Groups of male and female Sprague-Dawley rats [weight: 144-212 g; strain: Crl:CD(SD)IGSBR; 5 animals/sex/group] were orally gavaged with single dose of bis-POCPMPA at dose levels of 0 (vehicle control), 160 (low), 500 (mid) or 1500 mg/kg (high) followed by a 14-day observation period to investigate the potential acute toxicity in rats. Results: there were no deaths or drug related Clinical signs in the study. There were no drug related effects on body weight or food consumption. There were no effects on hematology, clinical biochemistry and urinalysis parameters. There were no treatment related macroscopic findings or Histopathological findings.

Comments: In this study, a dose level of 1500 mg/kg may be considered the NOAEL. Based on the body surface area factor, an equivalent dose in humans would be 243.5 mg/kg (14.6 g/day for a 60 kg person).

2. An acute oral gavage toxicity study of bis-POCPMPA in the beagle dog followed by 14-day observation period, Lot # 2454-A-2P, ClinTrials BioResearch LTD., Senneville, Quebec, December 7, 2000, (D990201/T4331-00023)

Groups of male and female beagle dogs [weight: 8.6-10.3 kg; 1 animal/sex/group] were orally gavaged with single doses of bis-POCPMPA at dose levels of 0 (vehicle control), 30 (low), 90 (mid) or 270 mg/kg (high) followed by a 14 days observation period to investigate the potential acute toxicity in dogs. Results: there were no deaths during the study. Clinical signs: were observed at the high dose animals and comprised primarily of emesis and salivation at 1 hr post dose. These signs were no longer apparent after the 2 hr post dose observation. There were

no drug related effects on body weight or food consumption. There were no effects on hematology, clinical biochemistry and urinalysis parameters. There were no treatment related macroscopic findings. Histopathology: drug related lesions were observed in the kidneys of both male and female animals (mid or high). The lesions were comprised of renal cortical tubular basophilia. At the high dose, minimal karyomegaly was also observed in animals.

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

Multiple dose toxicology-Tenofovir DF

1. A 14-day repeat oral gavage toxicity study of bis-POCPMPA in ICR CD-1 mice, Lot # 2454-A-2P, Gilead Sciences, Inc., Boulder, CO, January 1, 2001, (M990203/T4331-00017.1)

Groups of male and female ICR CD-1 mice [weight: 22.99-26.79 g; 5 animals/sex/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high) for 13 consecutive days. Results: one death occurred (high) male; a drug related effect could not be excluded. Clinical signs in the study. There were no drug related effects on body weight or food consumption. Hepatotoxicity was indicated by a mild, dose related rise of the mean serum ALT activity in all drug treated groups that was associated with an extremely mild increased incidence of single cell necrosis of the liver of male and female animals (high). Bone marrow toxicity was indicted by a reduction of the absolute neutrophil count in all treated groups and was non-dose-dependent and mild. Two female mice had marked reduction of their platelet counts (high). Histopathology: kidney toxicity was indicated by minimal proximal tubular epithelial karyomegaly in all males and one female (high).

Comments: The findings of this study indicate that the liver, kidney and bone marrow were the target organs of toxicity. In this study, a NOAEL could not be determined; it should be considered to be lower than 100 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be less than 8.11 mg/kg (486 mg/day for a 60 kg person).

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

Groups of male and female albino mice [weight: 21.9-32.3 g;

strain: Crl:CD-1(ICR)BR] were orally gavaged with bis-POCPMPA suspension (suspension vehicle lot # TX4331-99-01; dose volume = 10 ml/kg/day) at dose levels shown in Table 1 daily for a period of 13 consecutive weeks.

Table 1
Study design of the 13-week oral gavage toxicity study of tenofovir in the albino mouse

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

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

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

For toxicokinetic analyses, blood samples (approximately 1 ml) were taken at 0, 5, 15, 30 minutes, 1, 2, 4, 8 hr post-dose on days 1 and 91. The following organs were dissected free of fat and weighed: adrenal glands, brain, heart, kidneys, liver, ovaries/testes, spleen, thymus and uterus. A complete histopathologic examination was performed on the following tissues: abnormalities, animal identification, adrenals, aorta (thoracic), bone marrow smear (prepared from femur), brain (cerebellum, cerebrum, medulla), esophagus, eyes, Harderian glands, heart, intestine-large (cecum, colon, rectum), kidneys, liver, lymph nodes (bronchial, mandibular, mesenteric), ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (biceps femoris), skin with mammary gland, small intestine (duodenum, jejunum, ileum) spinal cord (cervical), 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: there were no clinical signs recorded during the study. Mortality: during the first few days of treatment, there were 13 unscheduled deaths in

animals at the 1000 mg/kg/day dose level. Prior to death, the animals had decreased activity and/or a swollen abdomen. On day 9, the dose level of the 1000 mg/kg/day was reduced to 600 mg/kg/day. The deaths seen at the 1000 mg/kg/day were considered due to problems administrating the test suspension. During the process of administering the test suspension, the gavage needle became blocked on a number of occasions, resulting in additional stress for the animal. It is for this reason that 600 mg/kg/day (60 mg/ml) is considered to be the highest dose level of tenofovir that may be practically administered to albino mice. Body weights: changes recorded for both males and females at all treatment levels were comparable to the controls and were considered to be unaffected by treatment with tenofovir. Food consumption: group mean food consumption for all treated groups was comparable to the controls and was considered to be unaffected by treatment. Ophthalmology: there were no effects of treatment. Hematology and clinical biochemistry: there were no effects of treatment. Organ Weights: there were no effect of treatment. Gross findings: no drug-related gross findings were noted in this study. Histopathology: findings associated with drug were seen in kidneys and duodenum. In the kidneys, a minimal tubular karyomegaly characterized by a slight enlargement of some nuclei was noted in all treated groups: low (4/15 females), mid (9/15 females and 6/15 males) and high (14/15 females and 8/15 males). In animals found dead during the study, an expected mild postmortem change often made the evaluation of nuclei difficult. In the duodenum, epithelial hypertrophy in the mucosa featured enlarged epithelial cell with increased amount of cytoplasm and was present in mid (3/15 males and 6/15 females) and high (13/15 males and 14/15 females) dose groups animals only.

Comments: A NOAEL for oral bis-POCPMPA in mice could not be identified; it should be considered less than 100 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be less than 8.11 mg/kg/day or 486.6 mg/day.

In this study, doses of tenofovir when administered by oral gavage to mice for a period of 13 weeks were well tolerated at dose levels of up to 600 mg/kg/day, the highest practical dose in albino mice and therefore 600 mg/kg/day would be suitable for future studies (ie., carcinogenicity study) of a longer duration.

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.

3. Five day repeated dose oral toxicity study of bis-POCPMPA in Sprague-Dawley male rats, Lot # 1074-191-A, Gilead Science, Inc., Foster City, CA, February 6 1997, (96-TOX-4331-002)

Groups of male Sprague-Dawley rats (4/group) were orally gavaged with bis-POCPMPA once daily at dose levels of 0 (vehicle control), 25 (low), 100 (mid) or 400 mg/kg/day (high) for 5 consecutive days. Results: no animal died or was sacrificed moribund prior to the scheduled sacrifice. No clinical signs of toxicity were observed during the treatment phase. Terminal body weights (9%) and body weight gains (4%) were reduced (high) only. A minor decrease in ALP activity was also present (high). No abnormal finding were observed at necropsy and no drug-related histopathologic effects were detected.

Comments: A target organ could not be identified in the study. The NOEL for oral bis-POCPMPA in male rats was 100 mg/kg/day. Based on a body surface conversion factor, an equivalent dose of oral bis-POCPMPA in humans would be 16.23 mg/kg/day.

4. A 14-day repeat oral gavage toxicity study comparing tenofovir DF and degraded tenofovir DF in Sprague-Dawley rats, Lots # GS-1616-31A-degraded tenofovir DF & GS-1616-31B-tenofovir DF, MPI Research, Inc. Mattawan, MI, February 23, 2001, (T433-00024/R2000081; GLP)

Groups of male and female Sprague-Dawley rats [strain: Crl: CD (SD)IGS BR; age: 5.5 weeks; 10 animals/sex/group] were orally gavaged at dose levels of 0 (vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) respectively with both lot # GS-1616-31A-degraded tenofovir DF and lot # GS-1616-31B-tenofovir DF for 2 consecutive weeks. Results: there were no deaths in the study. Clinical signs: no treatment related signs were observed. There were no drug related effects on body weight or food consumption. Gross or Histopathology: no changes were seen. No differences between treatments with either tenofovir or degraded tenofovir were noted.
Comments: A dose level of 300 mg/kg/day may be considered the NOEL for both tenofovir and degraded tenofovir in this study. Based on the body surface area factor, an equivalent dose in humans would be 48.7 mg/kg (2.9 g/day for a 60 kg person).

5. Four week oral gavage toxicity study of bis-POCPMPA in albino rats, Lot # 1156-27-13, Clinical Trials Bioresearch, Sennebille, Quebec, February 25, 1997, (96-TOX-4331-003)*

Groups of male and female Sprague-Dawley rats (10-18 animals/sex/group) were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 20 (low), 100 (mid) or 500 mg/kg/day (high) once daily for 4 weeks. For toxicokinetic analyses, blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose on days 1 and 28. Results: one animal was found dead on day 12 of the treatment period. Gross pathological

findings revealed a perforation of the esophagus. The death of this animal was not drug-related. There were no test article-related antemortem observation nor differences in body weight, body weight gains or food consumption data that were attributed to drug. The absolute and relative kidney weights were decreased significantly in males (high). Slight (less than 2-fold) increases in ALT values occurred in males and females (high) and slight decreases were seen in total protein, albumin and sodium values in females (high). Statistically significant changes in hematologic parameters occurred [increased in red cell distribution width and decreased hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration] in males (high) and decreased hemoglobin in females (high). There were no gross or histological findings related to the treatment.

Comments: A target organ could not be identified in the study. Bis-POCPMPA produced statistically significant changes in hematological parameter values; however, these values were within a historically acceptable range for animals of this age and strain. The NOEL for oral bis-POCPMPA in male and 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 16.23 mg/kg/day.

6. 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 albino rat, Lot # 4331-05-XA, Clinical Trials Bioresearch, Sennebille, Quebec, Canada March 10, 1998, (97-TOX-4331-002)

Groups of male and female Sprague-Dawley rats [Crl:CD(SD)BR; 10-18 animals/sex/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 30 (low), 100 (mid), 300 (high) or 1000 (very high) once daily for 13 or 42 weeks. Data from animals in the 42-week cohort for this study will be reported separately. For toxicokinetic analyses, blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose on days 1 and 85. Results: the treatment resulted in a dose-dependent increase in the incidence and severity of salivation in all treated groups. Statistically significant suppression of body weight gains and decreases in mean food consumption (male) values were observed in animals (very high). Hematological and biochemical parameters: statistically significant increases in segmented neutrophils (males), basophil (males), RBC counts (males), platelet, reticulocyte (females) and RBC distribution widths and decreased in hemoglobin (males), MCV, MCH and MCHC (females) values were observed in animals (very high). The test article induced statistically significant changes in clinical chemistry parameters that included mild to moderately decreased cholesterol (males) and triglyceride levels in animals (very high), marginal to minor increases in BUN, creatinine, AST

(females), ALT, ALP (males), phosphorus (males), sodium and chloride (males) values, and marginal to minor decreases in potassium and glucose (female) values in rats (very high). There were no treatment-related changes in the urinalysis parameters. Organ weights: statistically significant increases were seen in adrenal, gonad, brain and lungs weights relative to body weights (high and very high). Histological findings: related to the treatment were seen in the kidneys and gastrointestinal tract. 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). This was also seen to a lesser degree in all males (high). Some animals from both sexes (very high) exhibited epithelial hypertrophy in the duodenal mucosa, featuring enlarged epithelial cell width, occasionally decreased inter villus spaces and was occasionally described as a thickening of the duodenal wall. Gastritis (occasionally ulcerative) was noted in a few male and female animals (very high) and one male in the high dose group. Typhlitis (inflammation of caecum) characterized by light inflammation with mucosal atrophy was observed in the treated males (high and very high).

Comments: The NOEL for oral bis-POCPMPA in male and 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 16.23 mg/kg/day.

7. A 13 and 42-week oral gavage toxicity study (with a 13-week recovery period) of bis-POCPMPA in the albino rat, Lot # 4331-05-XA, Clinical Trials Bioresearch, Sennebille, Quebec, Canada, February 25, 1999, (97-TOX-4331-002)

Groups of male and female Sprague-Dawley rats [weight: 128-220 g; strain: Crl:CD(SD)BR; 20-27 animals/sex/group] were orally gavaged with bis-POCPMPA at dose levels of 0 (vehicle control), 30 (low), 100 (mid), 300 (high) or 1000 mg/kg/day (very high) once daily for 13 or 42 consecutive weeks followed by a 13-week (5 animals/sex/group) drug free recovery period (week 55). For toxicokinetic analyses, blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose on days 1 and at weeks 13, 26 and 42. 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: are summarized in Table 2. No drug-related mortalities occurred in this study. The treatment resulted in a dose-dependent increase in the incidence and severity of salivation in all treated groups. Statistically significant suppression of body weight gains and decreases in mean food consumption values were observed in animals (high and very high). By the end of the recovery period, the body weight gains and food consumption were comparable to the controls; however, the body weights (very high) remained lower than the controls. No drug-related ocular findings were observed. Hematological parameters: statistically significant increases in hematological parameters (very high) over the course of the study included increased white blood cell and segmented neutrophil counts, increased red blood cell counts, decreased hematocrit and related red cell parameters (hemoglobin concentration, MCV, MCH, and/or MCHC), increased red cell distribution width, and increased platelet counts and mean platelet volume (females). No consistent changes in hematology parameters were seen in the high dose group or less. No significant changes were observed in the recovery animals. Biochemical parameters: changes included: dose-related, slight to moderate decreases in cholesterol (males, very high); moderate to marked decreases in triglyceride levels in males and females (high and very high); slight increase (<2-fold) in ALT values in males (>mid) and females (very high); a slight increase (<2-fold) in AST values in males (very high); marginal to slight increases in phosphorus (very high); slight decreases in total protein and globulin levels (very high) and marginal to slight increases in A/G ratios in males (high and very high); slight to moderate decreases in serum bicarbonate in females (high and very high); and a marginal increase in creatinine in males and females (very high). No changes in serum chemistry parameters were observed at the end of the recovery period. Urinalysis: showed slight decrease in urinary pH and increases in amorphous urate crystals in males and females (very high). Organ weights: statistically significant increases were seen in adrenal, gonad, brain and lungs weights relative to body weights (high and very high). Gross Finding: drug-related thickening of the wall in the duodenum and jejunum or depressed areas in the stomach were seen. They occurred mainly in very high dose animals at the interim sacrifice (week 14). Thickening of the duodenum (mid, high and very high) and jejunum (males, very high) and depressed area in