leukopenia was seen for both sexes at the mid and high dose levels. A very slight but statistically significant reduction in mature neutrophils was noted in low dose females.

Treatment-related nephrotoxicity was indicated by significant increases in serum BUN and creatinine for high dose and mid dose males. Serum potassium was also decreased in those animals; sodium and chloride levels were generally unaltered. Urinalysis data showed increased leukocytes, slight proteinuria and slight glycosuria in males and females at mid and high dose levels, suggestive of renal toxicity. Organ Weights: administration of PMEA resulted in significant increases in absolute and/or relative kidney weights for high and mid dose animals. Pathology: treatment-related changes were found in kidneys, bone marrow, thymus and prostate. The kidneys were enlarged and pale. Microscopically, nephropathy was observed in all rats at mid and high dose levels. The primary site of injury was tubular epithelium, followed by glomerular and interstitial involvement. Bone marrow hypocellularity, represented by loss of cellular components (myeloid, erythroid and megakaryocytes) and replaced by fat, was seen for 9/10 males and 3/10 females at high dose, and 3/10 mid dose males. This lesion was primarily seen in the diaphysis. At necropsy, thymic gross lesions were seen in 7/10 and 2/10 mid dose males and females, respectively and 7/10 and 6/10 high dose males and females, respectively. Microscopically, lymphocyte depletion in the thymus was diagnosed in all high dose animals, all mid dose males and 9/10 mid dose females. Subacute suppurative inflammation of prostate was apparent in 5/10 high dose males.

Comments: The major toxic effects of sc administration of PMEA to rats for 30 days involved the kidneys and hemopoietic system. Nephrotoxicity was manifested by increased serum levels of BUN and creatinine, and decreased serum potassium in mid and high dose animals. The subacute suppurative inflammation in the prostate of high dose males might be secondary to kidney injury.

In general, toxicity was greater in males than females at their respective dose levels. On the basis of the present study, the low dose of (3 mg/kg/day) may be considered a NOAEL. Based on equivalent body surface area dosage conversion, the highest sc dose for humans would be 0.43 mg/kg.

21. Multiple-Dose Intravenous Pilot Study in Monkeys, ——41671 HPMPC, Lot # 26870-64 and ——-40085 PMEA, Lot # 26870-059, ——————— January, 1989, (——— 20954/88064)

Groups of male and female cynomolgus monkeys (weight: 2.4 - 4.9 kg; age: unknown, 1 animal/group) were administered either HPMPC

(0.1, 1.0, 10.0 or 50 mg/kg/day) or PMEA (1.0, 5.0, 10.0, 25.0 or 50 mg/kg/day) in 0.9% sodium chloride intravenously for 14 consecutive days via a saphenous vein in a total volume of <5.0 ml/kg at a rate of 0.1 ml/sec. Control groups received 0.9% sodium chloride for injection under identical experimental conditions. The objective of the study was to investigate the toxicity of HPMPC and PMEA and to select appropriate dose levels for the subacute IV toxicological evaluation to support an initial clinical trial in man. With HPMPC, significant nephrotoxicity (acute tubular necrosis) occurred at doses of 1.0 mg/kg/day or more. Ten or 50 mg/kg/day was lethal within 7 - 9 days and nephrotoxicity was considered to be the major contributing factor. Overt toxicity and death occurred after doses of 10 mg/kg/day or more. At the 10 and 50 mg/kg/day dose levels, decreased activity was apparent in both monkeys on the 7th day of treatment. The 50 mg/kg/day monkey was found dead the following morning while the 10 mg/kg/day monkey died after the 9th dose. Severe nephrotoxicity characterized morphologically by acute tubular necrosis was detected in both the monkeys. Significant elevations in creatinine and BUN were also detected prior to death. Other drug-related findings consisted of necrosis of mucosal crypt cells in the colon and/or duodenum at 10 and 50 mg/kg/day, pulmonary congestion along with intra-alveolar hemorrhage and edema, depletion of lymphocytes in lymphoid tissues, lymphopenia, and degeneration and necrosis of gastric mucosa of the stomach at 50 mg/kg/day. With PMEA, no drug-related toxicity at 1 mg/kg/day and slight swelling of hepatocytes at 5 mg/kg/day were observed in animals. At 10 mg/kg/day, no clinicopathologic evidence of toxicity was detected after 14 days of treatment. At 25 mg/kg/day beginning on 10 day of treatment, animals developed significant skin lesions. At 50 mg/kg/day, the animals developed significant epidermal lesions consistent with toxic epidermal necrolysis requiring an early sacrifice after 12 days of dosing. Lymphoid depletion and slight testicular degeneration were observed at this dose level. Marked inappetence and nephrotoxicity (vacuolar degeneration and dilatation of tubules) were also noted.

Comments: Based upon the results of these range finding studies, a dose of 1.0 mg/kg/day may be considered the NOEL for PMEA. Based upon the observation of toxicities at 25 and 50 mg/kg/day, a high dose between 10 and 25 mg/kg/day PMEA dose levels should be selected for subchronic nonclinical safety studies in monkeys.

22. Fourteen day repeated dose oral toxicity study and pharmacokinetics of GS0393 administered via gavage Cynomolgus Monkeys, Lot # 1965-BL-1, January 8, 1993, (2-M52/ — GSI-M52-92-146)

Three groups of normal male and female cynomolgus macaque monkeys

(weight: 3.7 - 4.4 kg males and 2.3 - 2.8 kg females; age: unknown; 2 animals/sex/group) were administered PMEA via oral gavage at dose levels of 3, 10 or 30 mg/kg/day in sterile water once daily for a period of 14 consecutive days. A control group received sterile water under similar experimental conditions. Blood samples were taken for pharmacokinetic determinations on day 1 and 14 at 0.5, 1, 2, 4, 6 and 24 hr following the treatment. This study was designed to investigate the toxicological potential and pharmacokinetic profile of PMEA when administered via gavage to monkeys.

All animals survived to the scheduled sacrifice. There were no clinical observations nor physical examination findings suggestive of a test article-related effect. Individual body weight profiles indicated that all monkeys either gained or maintained their initial body weight when compared to the body weight taken at euthanasia. Evaluation of clinical hematology, serum chemistry and individual urinalysis data failed to reveal any abnormalities or changes when values from pretreatment periods were compared to day 15 values or when values from the treated groups were compared to respective control values. Organ weights: individual abnormalities included unusually low absolute and relative testes weights in one low and one mid dose male, low absolute and relative thyroid gland weights in one low and one high dose male, and one high dose female with an unusually high thyroid gland weight. Histomorphologically, the female revealed follicular hypertrophy possibly associated with colloid goiter.

Comments: Data generated from the daily oral administration of PMEA for 2 weeks to male and female monkeys suggest that a dose level of 30 mg/kg/day may be considered a NOEL. Based on equivalent body surface area dosage conversion, an equivalent dosage for humans would be 10 mg/kg/day.

23. One-Month Intravenous Toxicity Study in Cynomolgus Monkeys, 40085 (PMEA), Identification # 27337-21, December 28, 1989, (21456, 124003)*

Three groups of normal male and female cynomolgus macaque monkeys (weight: 2.9 - 3.3 kg males and 2.7 - 3.8 kg females; age: unknown; 2 animals/sex/group) were administered PMEA by intravenous injection (via saphenous vein) 1, 5 and 25 mg/kg/day in sterile saline (1.0 ml/kg and a dose rate of 0.1 ml/second) for a period of 30 - 31 consecutive days. A control group received sterile saline under similar experimental conditions. This study was designed to investigate the toxicological potential of PMEA when administered intravenously to monkeys in a one month sub-chronic toxicity study. Survival, Clinical Observation and Ophthalmoscopic Examination: All animal survived to the scheduled sacrifice. At the first weekly physical

examination, at the time of dosing or 30 min to 2 hr following dosing, one male (high dose) had hair loss, exfoliation, redness and scabbing on both forelimbs. Both females (high dose) had scabbing on the forelimbs, hind limbs and on the ventral thoracic area; one female had hair loss, exfoliation and redness on the forelimbs and hind limbs. Excreta findings such as soft stool, mucoid feces, diarrhea or decreased defecation were observed in all dose groups during the treatment period. The ophthalmological examination performed during study week 3 did not reveal any oculopathic lesion indicative of a toxic effect. Food Consumption and Body Weights: Decreased food intake was observed on day 0, 3 and 7 in the high dose group of animals; one female had decreased food intake on 20 of 31 days during the study period. No effect on food intake was observed in the low and mid dose groups. Body weight change for the overall treatment period (day 0-28) was lower in the high dose group male (43 g) than the control group (185 g); the females (high dose) had overall body weight loss (85 g) compared to their initial body weights, while the females (control) had overall body weight gain (115 g). Hematology, Serum Chemistry and Urinalysis: Mean lymphocyte count was decreased ($p<0.05$) in the males (mid dose). At the week 4 evaluation, the mean glucose level was decreased ($p<0.05$) in the males (mid dose). The mean potassium level was increased ($p<0.05$) in the males (high dose) prior to the termination of the dosing. The urine of one male (high dose) registered at +2 (slight to moderate) for glucose [a qualitative test on the scale of 1+ (trace to slight) to 4+ (positive)]. Aside from the kidney, lesions that appeared to be treatment-related in the high-dose group were found in bone marrow, lung, lymphoid tissues in males and in thymus in females. The lung lesion consisted of minimal to mild alveolar edema. Lymphoid atrophy involving the lymph node, spleen and/or thymus was noted in 3 of 4 animals at this dose. Macroscopic and Microscopic Examination: At the gross necropsy, animals had scabbing on various skin surfaces (high dose); these findings corresponded to the clinical observation noted for the animals during the treatment period. Histopathological examination of the skin tissues (high dose) revealed suppurative inflammation and/or hyperkeratosis. Organ Weights: No treatment-related effect was observed in organ weight values.

Comments: As evident from the dermal reaction (hair loss, exfoliation, redness and/or scabbing) and supported by the gross necropsy and microscopic findings, the primary target organ is the skin for the systemic toxicity of the test compound in the monkeys. A dose of 5.0 mg/kg/day of PMEA may be considered the NOAEL in this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 1.7 mg/kg/day. Additionally, the occurrence of glucose (+2, slight to moderate) in the urine of a high dose male, though not supported by abnormal histopathological findings, suggests that kidney is

one of the susceptible organs as it has been seen in the previous study in rats.

24. Thirty day repeated dose subcutaneous toxicity study and pharmacokinetics of GS0393 administered to Cynomolgus Monkeys, Lot # 1965-BL-2, [redacted] April 2, 1993, (2-M58/[redacted]GSI-M58-93-28)

Three groups of normal male and female cynomolgus macaque monkeys (weight: 4.6 - 4.9 kg males and 2.6 - 2.7 kg females; age: unknown; 3 animals/sex/group) were administered PMEA by a subcutaneous injection at 3, 20 or 40 mg/kg/day in sterile water for a period of 30 consecutive days. A control group received sterile water under similar experimental conditions. This study was designed to investigate the toxicological potential of PMEA when administered sc to monkeys in a one month sub-chronic toxicity study. Survival and Clinical Observation: one male (high), and one male and female (high) were euthanized in extremis on day 18 and day 30, respectively. All other animals survived to the scheduled sacrifice. The most remarkable findings were those related to generalized skin where increased pigmentation and fissures with sloughing of the superficial layers of the skin was noted in mid and high dose groups. The severity of the skin lesions appeared to be greater in the males than in the females. Hunched posture was related to the discomfort of the desquamation of the skin and the reluctance of the animal to move. Food Consumption and Body Weights: in general, males and females (mid and high) ate less food as compared to the other groups of animals. Males and females (mid and high) displayed a loss in the mean body weight over the course of the study. Physical Examinations: notable findings are summarized in Table 5.

Table 5
Summary of Notable Physical Examination Findings

Sex	Group	Finding
1 male	low	scrotum slightly enlarged
1 male	mid	scrotum moderately enlarged
1 male	mid	alopecia/flaking at injection site
1 male	mid	flaking skin at the sites, glans penis swollen
1 male	high	urine stains, flaking skin at the sites, peeling skin over eyebrows, chin & throat,
1 fem.	low	scrotum moderately swollen
1 fem.	mid	mild skin scale over the sites
1 fem.	mid	skin with flaking and crust at the sites

1 fem.	high	skin scaly & with crust at the sites
1 fem.	high	animal thin, skin scaly & crusty at the sites
		skin with scale & crust over the sites

Clinical Pathology-Hematology, Clinical Chemistry and Urinalysis: the absolute and percent monocyte values (low) on day 14 were significantly elevated. Statistically significant depression (mean serum phosphate) and a significant elevation (mean serum chloride) were noted in high dose animals on day 14. In low and high dose males, mean serum creatinine values and mean ALP (high) were significantly elevated compared to controls on day 31. In mid and high dose females on day 14, the mean serum GGT values were depressed on day 14 and 31. Urinalysis data were unremarkable. Organ Weight Observations: treatment-related significant alterations in organ weight values were observed for liver and kidney. Relative liver weights were increased for high dose males. Mean absolute kidney weights were increased for high dose males and kidney to final body weight values were increased for high dose females. Gross Pathology Observations: treatment-related gross changes were observed at necropsy in skin and kidney. Treatment-related skin lesions were observed primarily on the dorsum in mid (2 males) and high (2 females) dose animals. These lesions consisted of a single or multiple pale areas in which there were also concurrent desquamation of superficial layers of epidermis. Black pigmentation of the face was observed in all high (females), mid (1 male), high (1 male) and mid (1 female) dose animals. Unusually pale kidneys were observed in 2 females and all male (high) dose groups. Histopathologic Observations: treatment-related changes revealed an increased incidence of enteropathy in the large and small intestines of mid (males) and high (males and females) dose animals. Other apparent test article-related lesions included lymphoid depletion in the spleen of mid and high dose males, and high dose females; decreased zymogen granules in the pancreas of high dose males and females; decreased cytoplasmic vacuolization in the adrenal cortex in high dose males; an increased incidence of nephropathy in the kidney and cellular depletion in the bone marrow of high dose males and females; lymphoid depletion in the mandibular and mesenteric lymph nodes of high dose males and females; chronic inflammation: in mid and high dose males and females, epidermal atrophy and necrosis in high dose males and females and increased melanin in mid (males) and high (females) dose groups; and epithelial necrosis: (mid and high dose males), epidermal atrophy (mid and high dose males and high dose females) and chronic inflammation (mid and high dose males, low, mid and high dose females) at the injection site's skin.

Comments: Daily sc administration of PMEA (3 mg/kg/day) was associated with minimal but detectable changes similar to those seen with greater severity in the animals administered 20 or 40 mg/kg/day. Under these test conditions, a NOEL could not be identified.

It is apparent from the clinical, gross necropsy observations and histomorphologic examinations that the skin was a primary target organ displaying manifestation of PMEA toxicity in monkeys. Clinical observations of affected animals revealed a tightening and/or shrinkage of the skin with subsequent tearing and sloughing of the superficial layers of the epidermis. Chronic inflammation was also observed in the injection site skin of low dose animals. Participation of an immunologic reaction leading to the skin toxicity could not be ruled out.
With regard to kidney as a target organ, although there was no biologically relevant elevations in the clinical chemistry values noted, significant changes in the kidneys (histomorphological) point to the fact that kidney may be another target organ. Lymphoid depletion in the spleen and lymph nodes, and cellular depletion of the bone marrow suggested the drug was immunotoxic.

25. A ninety-one day repeated dose intravenous or subcutaneous toxicity study and pharmacokinetics of PMEA administered to cynomolgus monkeys, Lot # 393B93-01, June 29, 1994, (93-TOX-0393-001, -2-Q58)

Groups of male and female cynomolgus monkeys (6 animals/sex/group) were administered PMEA by a subcutaneous or intravenous injection at 0 (vehicle control; both sc and iv; #1), 3 (low; iv; #2), 8 (mid; iv=6/sex; #3)/(mid; sc=6/sex; #4) or 20 mg/kg/day (high; iv; #5) in sterile saline for a period of 91 consecutive days. Two monkeys/sex/group were assigned to a 28-day non-treatment recovery period following the treatment. Survival and Clinical Observations: one male (#5) was euthanized on day 7 due to a leg fracture and one male (#5, recovery) was euthanized on day 18 due to his poor health. All other animals survived until the scheduled termination of the study. Clinical observations included hunched posture, emaciated/thin appearance, dehydration, ruffled coat and pale mucous membranes (#5 males only). There was a greater incidence of pigmentation of the skin (#2,3,4,5) as compared to the controls. At the administration site, there was a greater incidence of dry, flaky skin, ulceration, erythema and swelling in the treated animals compared to the controls. During the recovery period, the incidence and severity of clinical signs generally decreased especially those associated with the skin. Food Consumption and Body Weights: males (#5), lost 0.1 kg weight during the treatment and gained 0.3 kg during the recovery period. Females (#5) lost 0.2 kg

during the treatment period and gained 0.2 kg during the recovery. There were no apparent differences in mean food consumption values when treated animals were compared with the controls. Clinical Pathology-Hematology, Clinical Chemistry and Urinalysis: on day 50, the mean erythrocyte counts and hemoglobin and hematocrit values (#5) were reduced as compared to the mean control values; the hematocrit value was significantly depressed. These hematology values indices were also reduced (#5) on days 92 and 120. There was a significant elevation in the mean absolute and percent polymorphonuclear leukocyte values and a significant depression in the percent lymphocyte values on day 50 (#5), as compared to the control values. Statistical evaluation of the mean serum chemistry values in males revealed significant elevation in creatinine value (#4,5; day 22), total protein value (#5, day 50), calcium and sodium values (#4,5; day 50), chloride value (#4,5; days 50 and 92). In males (#5), A/G value was significantly less than the control value on day 92. In females (#5), mean creatinine (day 92) and potassium (days 22 and 50) values were significantly elevated. There were no relevant differences in urinalysis data for the males or females at any of the time points evaluated. Organ Weight Observations: evaluation of the mean organ weight data revealed a significant elevation of the adrenal gland-to-final body weight ratio (#5, male) and in the kidney-to-final body weight ratio (#5, males and females). There were no significant organ weight differences in the animals following the recovery period. Gross Pathology Observations: treatment-related gross necropsy findings were observed in the kidney, skin and administration site. In the skin, there was an increased incidence of dark pigmentation in all treated animals as compared to the controls, which persisted following the recovery period. There was an increased incidence of foci on the skin noted most commonly on the forelimbs, hindlimbs, feet, neck, tail or forehead (#3,4,5 males; #5, females). Erythema, swelling and/or ulceration of the skin, primarily of the penis, was observed in the majority of males (#3,4,5). Unusually pale kidneys (1-2/group) were noted in terminal sacrifice (#1,3,4,5 males and #5 1 female) and in one male monkey each in recovery groups 4 and 5. The pale color of the kidneys (#5 both terminal and recovery) males was caused by treatment-related nephropathy. Histopathologic Observations-Kidney: treatment-related alterations of the renal cortex in the form of a mild to moderate tubular nephropathy was observed in kidneys in 5/6 males (#5), in 1/4 females (#5) and 1/1 recovery males (#5). In the recovery male, tubular nephropathy was graded as moderate. The nephropathy was characterized by a number of alterations in proximal convoluted tubules of the cortex. Microscopic changes observed included karyomegaly of tubular cell nuclei, atrophy and decreased cytoplasmic density of tubular cells and by occasional individual tubular cell necrosis with sloughing of shrunken eosinophilic necrotic cells into the tubular lumen. The

nephropathy was not observed in kidneys of monkeys in any other dose group. In the skin of the face, there was an increased incidence and severity of pigmentation in males and females (#2,3,4,5). The severity of the increased pigmentation was dose related and ranged from minimal to moderate. Acanthosis was noted in males (#5) and females (#2,3,4,5) and chronic inflammation was noted (males, #5 and females, #3,4,5). There were apparent test article-related lesions consisting of ulceration, acanthosis, chronic inflammation and increased melanin. These lesions were generally noted as minimal to moderate and were somewhat dose-related in degree of severity in males and females (#2-5). The degree of severity generally decreased over the recovery period; no ulcers were noted for any animal following the recovery phase. Lesions at the administration site included perivascular hemorrhage, noted in the majority of the animals (#1,2,3 and 5); acute inflammation, noted in equal incidence (#1,2,3,5 males and #1,2 females); chronic inflammation, noted in some #2-5 males and #1-5 females; acanthosis, noted in #3-5 males and females; and increased melanin noted in males and females (#2,3,4,5). By the end of the recovery period, acute inflammation was not observed at the administration site in any of the dose groups and chronic inflammation was restricted in incidence to #5 animals. Perivascular hemorrhage (#1,3,5 males and females) and acanthosis (#3,5 males and #3,4,5 females) and increased melanin (#3,4,5 males and females) were still present to a remarkable degree.

Comments: Based on the data generated during this study, PMEA appeared to preferentially affect the skin. A NOEL could not be determined [less than 3 mg/kg/day]. Kidney and skin are identified as the target organs. In regards to the systemic effects of PMEA, other than those noted in the skin, the NOAEL is 8 mg/kg/day. In a comparison between the iv (#3) and the sc (#4) routes of administration of 8 mg/kg/day PMEA, there did not appear to be any apparent differences in the incidence or severity of changes seen. Both groups of animals displayed approximately the same level of skin involvement and hyper-pigmentation and neither group of animals displayed indications of systemic toxicity.

In the treated animals on day 50, there were significant differences in values of various clinical pathology-hematology and chemistry parameters. However, the differences appeared to be subsided by day 92 of the treatment and it appeared that the animals might be recovering from those changes. Therefore, the changes on day 50 of the treatment might be transitory.

V. GENETIC TOXICOLOGY:

Summary of genetic toxicology studies:

1. **Mutagenicity test on bis-POM PMEA in the in vivo mouse micronucleus assay, Lot # 2166-A7P, [redacted] October 29, 1997, (T0840-00017, [redacted] 18760-0-455)***

2. **Mutagenicity test on bis-POM PMEA in the L5178Y TK$^{+/-}$ mouse lymphoma mutation assay, Lot # 2166-A7P, [redacted] January 15, 1998, (T0840-00018, [redacted] 18760-0-431)***

Genetic toxicology-Adefovir

3. **Ames Microbial Mutagenicity Assay and E. Coli WP2 uvrA Reverse Mutation Assay, [redacted] 40085 (PMEA), Lot # S89B021, [redacted] February 21, 1990, [redacted] 21204/89036)***

4. **Human Peripheral Blood Lymphocyte Clastogenesis Assay, [redacted] 40085 (PMEA), Lot # S89B021, [redacted] September 27, 1991, [redacted] 21472/89051)***

Review of genetic toxicology studies:

1. Mutagenicity test on bis-POM PMEA in the in vivo mouse micronucleus assay, Lot # 2166-A7P, [redacted] October 29, 1997, (T0840-00017/ [redacted] 18760-0-455)

Groups of male and female mice [Crl:CD-1 (ICR) BR; 5 animals/sex/group/harvest timepoint] were administered adefovir dipivoxil via oral gavage at dose levels of 0 (vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg/day (high) to evaluate clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in bone marrow. The animals were euthanized at approximately 24, 48 and 72 hr after dosing for extraction of the bone marrow. Results: one male mouse (high, 72 hr harvest timepoint) was found dead at approximately 45.8 hr after dosing. All other animals appeared normal immediately after dosing and remained healthy until the appropriate harvest timepoints. Bis-POM PMEA was cytotoxic to the bone marrow (statistically significant reduction in the PCE:NCE ratio) at all dose levels in both sexes at the 48- and 72-hr harvest timepoints. Bis-POM PMEA induced statistically significant increases in micronucleated poly chromatic erythrocytes over the dose range tested. Conclusions: bis-POM PMEA was found to be cytotoxic and clastogenic in the bone marrow in the mouse micronucleus assay.

2. Mutagenicity test on bis-POM PMEA in the L5178Y TK$^{+/-}$ mouse lymphoma mutation assay, Lot # 2166-A7P, [redacted] January 15, 1998, (T0840-00018/ [redacted] 18760-0-431)

Bis-POM PMEA was tested in the L5178Y TK$^{+/-}$ mouse lymphoma cell line at concentrations of 1.57, 3.13, 6.25, 12.5 37.5 or 50.0 µg/ml in the presence or absence of rat liver S9 metabolic activation to evaluate the ability of bis-POM PMEA to induce forward mutations at the thymidine kinase (TK) locus. Results: under non-activation conditions, moderate cytotoxicity was induced at 1.57 µg/ml, and the 3.13 µg/ml treatment was highly cytotoxic. All other concentrations were too cytotoxic. All concentrations (1.57 - 50.0 µg/ml) induced mutant frequencies that exceeded the minimum criterion for a positive response. Under activation conditions, bis-POM PMEA induced dose-related cytotoxicity. All concentrations (1.57 - 50.0 µg/ml) induced mutant frequencies that exceeded the minimum criterion for a positive response. Conclusions: under the conditions of this assay, bis-POM PMEA was found to be a positive mutagen in the presence or absence of metabolic activation.

3. Ames Microbial Mutagenicity Assay and E. Coli WP2 uvrA Reverse Mutation Assay, [redacted] 40085 (PMEA), Lot # S89B021, [redacted] February 21, 1990, [redacted] 21204/89036)*

An Ames microbial mutagenicity assay and an E. coli reverse mutation assay were performed to determine the potential of PMEA to induce base pair substitution of frameshift mutations in Salmonella typhimurium (his-) strains and E. coli strains. The compound was tested with and without exogenous metabolic activation using the S-9 fraction of a rat liver homogenate in the mutation assay at five nominal concentrations (312.5, 625, 1250, 2500 and 5000 µg/plate. In conclusion, the results indicated that PMEA is not mutagenic in the Ames microbial mutagenicity assay or the E. coli reverse mutation assay up to a concentration of 5000 µg/plate.

4. Human Peripheral Blood Lymphocyte Clastogenesis Assay, [redacted] 40085 (PMEA), Lot # S89B021, [redacted] September 27, 1991, [redacted] -21472/89051)*

Based on the results of a preliminary assay with no metabolic activation in which human lymphocyte cultures were PMEA-treated at levels of 1.56 - 200 µg/ml for 48 hr, levels of 6.25, 12.5, 25 and 50 µg/ml were selected to determine the potential of PMEA to induce chromosomal aberrations. Appropriate positive and negative controls were included; ganciclovir, an anti-viral agent, was used as reference compound. In the full assay, human peripheral

blood lymphocytes from 2 donors were exposed to PMEA for the last 48 hr of a 72-hr culture period. A total of 400 metaphase cells for each dose group were examined microscopically for aberrations. The results of the chromosome aberration analysis indicate that PMEA exhibits a statistically significant clastogenic effect in the human peripheral blood lymphocyte chromosome aberration assay at the 25 and 50 µg/ml level tested. The numbers of aberrant chromosomes from culture flasks receiving PMEA at 6.25 and 12.5 µg/ml dose levels were elevated in a dose related manner relative to the negative control (statistically not significant). The reference agent, ganciclovir at 50 µg/ml, induced a slightly lower frequency of chromosome breakage than the comparable 50 µg/ml dose of PMEA. The positive control, Mitomycin C at 0.05 µg/ml was clastogenic causing greater than a seven-fold increase in the frequency of cells with chromosome aberrations. The untreated and solvent (0.1N NaOH) controls were not statistically different from each other and were within the expected ranges for this assay.

Comments: PMEA exhibited a significant clastogenic effect at the two highest dose tested. At these dose levels, the mitotic index was depressed to approximately one-half of the negative control value. At the two lower dose levels, there were increases in the frequencies of chromosome aberrations which appear to be dose-related. Thus, all four dose levels of PMEA tested increased both the percentage of damaged metaphases and the number of aberrations per cell in a concentration dependent manner. PMEA proved to be more clastogenic than the reference agent, ganciclovir.

VI. CARCINOGENECITY:

The following study was conducted in accordance with the FDA Good Laboratory Practices Regulations.

Adefovir dipivoxil: oral (gavage) oncogenicity study in the albino mouse, Lot # TX840-98-02, November 5, 2001, (98-TOX-0840-005)

Groups of male and female albino mice (Crl:CD^{R}-1(ICR)BR; age: 6 week; weights: 21-30.7 g for males and 17-24.9 g for females) received adefovir dipivoxil via gavage (dose volume=10 ml/kg/day) using a stainless steel gavage tube. Each dose was based on each animals most recent body weight. Animals were dosed at 0, 1, 3 or 10 mg/kg/day approximately the same time of day, 7 days a week for a period of 104 weeks according to an experimental design shown in Table 1. A standard commercial laboratory diet (

certified Rodent Chow —— from ———————→ was freely available to the animals. Animals were randomly assigned to each of the 4 treatment groups. Male and females were randomized separately. The mice were housed in individual suspended wire-bottom stainless steel cages, and arranged on each cage-truck in a random block design, balanced for sex in that alternating rows were of opposite sex. Cage waste tray papers were routinely changed three times a week and cages and cage-trucks were changed approximately every other week. Environmental conditions were monitored and controlled: temperature 22 ± 3°C; light cycle 12 hr light and 12 hr dark and humidity in the range of 50 ± 20%. All animals were observed for signs of ill health or response to treatment once daily, with a second daily observation for dead or moribund animals. From week 26 onward, all animals were examined for the presence of palpable masses. For toxicokinetics, blood samples were taken on days 1 and 180 at 0, 15, 30 min and 1, 2, 4, 8 and 24 hr post dose; plasma samples were analyzed by a validated —— method. A complete histopathologic examination was performed on the following tissues: gross 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), gall bladder, Harderian glands, 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 and vagina.

Table 1
Experimental design of the oral oncogenicity study in mice

Group/ treatment (mg/kg/day)	Number of mice			
	Main study		Toxicokinetic study	
	male	female	male	female
vehicle control	60	60	-	-

1 (low)	60	60	-	-
3 (mid)	60	60	-	-
10 (high)	60	60	48	48
health screen	10	10	-	-

Results-mortality: animals that died during the study are shown in Table 2. For males and females (low or mid) the mortality rate observed was comparable to the controls. A portion of mice of both sexes died or were killed pre-terminally in a moribund condition and were subsequently examined at necropsy and also examined histologically. As is common in mice of this strain and age range, lymphoid, pulmonary and hepatocellular tumors were the most frequent neoplastic factor that were considered to be contributory to the deaths of these animals with neoplastic lesions in general, and lymphoid tumors in particular, being more common in female than in male animals. No treatment related effects were discerned in the distribution of specific neoplastic lesions, or associated groups of neoplastic lesions, as causes of death of preterminal decedent animals. The mortality rate was higher than the controls in the high dose group of animals.

Table 2

Mortality at termination (week 104) of the mice oncogenicity study

Group/treatment (mg/kg/day)	males		females	
	mortality	%	mortality	%
vehicle control	26	43	22	37
1 (low)	27	45	27	45
3 (mid)	27	45	28	47
10 (high)	41	68	32	53

Clinical signs and mass palpations: no drug-related clinical signs were observed throughout the duration of the study. Incidental findings included but were not restricted to thin fur, scabs, fur staining, ungroomed fur, pallor of the skin, prominent back bone, dehydration, broken teeth and decreased activity. The number of mice which developed cutaneous or subcutaneous massed during the treatment period was 26. The distribution of animals with masses observed at necropsy is shown in Table 3.

Table 3

Distribution of animals with masses at necropsy

Group/treatment (mg/kg/day)	Total number of mice with masses	
	male	female
vehicle control	3	4
1 (low)	4	2
3 (mid)	4	2
10 (high)	3	2

Body weight and food consumption: are shown in Tables 4, 5 and 6. Generally, throughout the treatment period, the group mean body weights or body weight gains of the treated mice were comparable to the concurrent control group. Weekly group mean food intake values of the treated mice were generally comparable to the controls.

Table 4
Group mean body weights (g) during the mice oncogenicity study

Dosage Group (mg/kg/day)	group mean body weights (g)							
	week 0		week 35		week 70		week 104	
	male	female	male	female	male	female	male	female
Vehicle control	26.81	21.56	37.54	31.21	39.33	33.32	37.07	32.92
1 (low)	26.45	21.04	37.86	31.64	38.75	33.16	37.12	34.25
3 (mid)	26.65	22.61	38.15	31.18	39.03	34.04	38.21	33.96
10 (high)	26.46	22.37	37.21	30.4	38.45	32.92	38.92	32.41

Table 5
Body weight gains (g), summary of means

Dosage Group (mg/kg/day)	body weight gains (g)							
	wk 0-26		wk 26-52		wk 52-78		wk 78-104	
	male	female	male	female	male	female	male	female
Vehicle control	10.18	9.10	1.56	1.27	0.14	1.42	-1.16	0.01
1 (low)	10.55	10.27[b]	1.73	1.1	-0.31	1.36	-1.22	0.96
3 (mid)	10.82	9.64	1.21	1.35	-0.25	1.79	-0.67	0.61
10 (high)	10.33	9.11	1.79	1.4	0.12	0.78	-0.10	0.12

b= P<0.01 (Dunnett's)

Table 6

Group mean food consumption (g/animal) during the mice oncogenicity study

Dosage Group (mg/kg/day)	group mean food consumption (g/animal)							
	week 0		week 35		week 70		week 104	
	male	female	male	female	male	female	male	female
Vehicle control	43.2	37.8	40.4	41.8	39.2	40	40.8	41.6
(low)	42.3	38.8	40.1	42.1	40	40.5	43.1	41.7
3 (mid)	44	36.9	40.3	40.6	40.3	38.8	43	43.1
10 (high)	43.4	35.3[b]	39.7	39.9	40.5	38.5	46	41.1

b = $p<0.01$ (Dunnett's)

Hematologic and clinical chemistry determinations: no drug-related changes were observed.

Toxicokinetics: the results are shown in Table 7. All animals appeared normal for the entire study period. No substantive changes in the pharmacokinetics could be seen between the first and the 180th dose. The were no apparent differences between males and female in the study.

Table 7

Mean Pharmacokinetic parameters for adefovir dipivoxil dosed to mice via gavage (10 mg/kg/day) for 104 week

Parameters	Day 1		Day 180	
	male	female	male	female
Cmax (µg/ml)	0.741	0.758	0.486	1.09
Tmax (hr)	0.25	0.25	0.25	0.25
T½ (hr)	4.89	2.87	8.87	6.84
$AUC_{0-\infty}$ (µg*hr/ml)	1.94	1.60	2.45	2.79

Gross findings: a number of gross lesions, primarily affecting the urogenital tract of male animals showed an increased

incidence with increasing dosage of the test article. The incidence of gross findings is shown in Table 8. It was apparent that with increasing dosage of adefovir dipivoxil there was an increased tendency for male mice to develop urinary tract obstructions, either pelvic cavity masses or penile ulceration. The etiology of this change is undetermined.

Table 8

Treatment related or possibly treatment related gross changes observed at necropsy (male mice)

Organs/tissues	mg/kg/day			
	controls	1 (low)	3 (mid)	10 (high)
No. of mice examined	60	60	60	60
Penis protrusion	3	6	10	30
Skin scab	4	9	5	15
Seminal vesicle enlargement swelling	2 -	1 -	3 1	4 5
Pelvic cavity mass	3	6	2	8
Ureter dilatation thickening	12 3	12 5	16 9	32 10
Kidney pelvic dilation	5	7	9	11

Histopathology:

Neoplastic/proliferative changes: a slight increase was observed in the incidences of proliferative lesion of the *pars distalis* of the pituitary gland (adenohypophysis) in female (high), but not in the male animals of this group. The incidence of these changes is shown in Table 9.

Table 9

Incidence of treatment related pituitary gland proliferative lesions (female mice)

Lesions	mg/kg/day			
	controls	1 (low)	3 (mid)	10 (high)
No. of mice examined	57	59	59	59
Hyperplasia: pars distalis	3	2	2	3

Adenoma: pars distalis	3	3	3	6
Carcinoma: pars distalis	-	-	-	1
Total proliferative lesions	6	5	5	10

Statistical analysis of these incidences by Peto's survival-adjusted tend test indicated a significant treatment associated effect for the incidence of adenomata in the pituitaries of female animals (p=0.04) where as Peto's one-tailed test did not indicate a statistically significant increased for the incidence of tumors in treated group when compared to the control group. For a common tumor, the incidence is not considered significant.

Comments: To put this into context, it should be noted that untreated female control mice from a series of previously completed studies in the laboratory showed spontaneous incidences of pituitary adenomata ranging from 1/120 to 5/120 with an overall incidence of 2.5% while that of carcinomata ranged from zero to 2/120, overall 0.6%. In the published literature, incidences of 3.4% for adenomata and 0.2% for carcinomata have been reported in female mice while a combined incidence of 4.9% for both adenomata and carcinomata has also been reported.

Non-neoplastic changes: are shown in Tables 10 and 11. In the urinary tracts of mice of both sexes, but especially in male mice, a number of urogenital tract changes were observed that were considered to be associated with the treatment. These changes were generally inflammatory and/or degenerative in character and probably associated with an impediment to normal urine flow, with consequential changes being seen that are characteristic of increased urinary tract pressure such as urinary bladder dilatation and dilatation of renal tubules. These changes generally correlated with the gross changes. It was concluded that a treatment related change was present at the high dose level and that this change comprised tubular lesions within the kidney (Table 11), with consequential inflammatory changes throughout the male urinary tract, urinary bladder transitional cell hyperplasia, with other lesions occasionally affecting the prostate and seminal vesicles. A predominance of penile protrusion and genital/perigenital inflammatory/ulcerative lesions was also considered to be associated with this process.

Table 10
Incidence of treatment related renal changes

Renal changes	mg/kg/day							
	control		1 (low)		3 (mid)		10 (high)	
No. of mice examined	♂ 60	♀ 60	♂ 59	♀ 60	♂ 60	♀ 60	♂ 60	♀ 60
Cortical tubular karyomegaly	-	-	3	-	8	7	9	16
Tubular basophilia	7	4	7	5	4	5	12	13
Interstitial inflammation	7	20	7	6	10	5	17	4
Pyelitis/pyeloneph ritis	-	-	-	-	2	1	8	1
Dilatation: tubular	4	10	6	7	7	12	12	8
Deposits of pigment	-	-	2	3	5	3	4	8
Tubular mineral foci	-	-	-	-	1	-	14	1

Table 11

Incidence of treatment related urogenital tract lesions (male mice)

Lesions	mg/kg/day			
	controls	1 (low)	3 (mid)	10 (high)
No. of mice examined	60	40	45	60
Prostate inflammation	3	6	4	21
Seminal vesicles inflammation	1	3	3	11
Skin crust/scab ulceration	2 5	2 10	1 10	5 19
Urinary bladder dilatation hyperplasia inflammation	12 2 3	10 2 2	15 4 2	31 11 10
Spleen hematopoiesis lymphoid atrophy	38 3	22 4	21 6	48 18
Pelvic cavity bulbourethral gland hemorrhage				

hemorrhage	2	6	5	9

Comments: The oncogenicity potential of adefovir dipivoxil was investigated in mice at dosages of 0, 1, 3 or 10 mg/kg/day in comparison with untreated controls for a period of 104 weeks. The study protocol was approved by the ECAC. A slightly increased, but not statistically significant, incidence of adenomata of the pituitary adenohypophysis was present in female animals at the high dose level. No such effect was observed male animals.

Adefovir dipivoxil was not oncogenic in mice. The human AUC at a dose level of 10 mg/day (the proposed clinical dose) was approximately 0.2 µg*hr/ml. The average systemic AUC measured at the high dose level of 10 mg/kg/day during week 26 was 2.62 µg*hr/ml for the mice. Thus, the mouse: human AUC ratio was approximately 13 or drug exposure in the mice was 13-fold greater than that of the clinical dose.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Reproductive and developmental toxicology studies summary:

1. **Oral (gavage) fertility and general reproduction toxicity study of adefovir dipivoxil in rats, Lot # TX-840-96-03B, [redacted] May 16, 1997, (96-TOX-0840-001)***

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

3. **Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of adefovir dipivoxil in rats, including a postnatal behavioral/functional evaluation, Lot # TX-840-97-01B, [redacted] October 23, 1997, (97-TOX-0840-001)***

4. **Oral (stomach tube) developmental toxicity study of bis-POMPMEA in Rabbits, Lot # TX840-95-05, [redacted] March 27, 1996, (94-TOX-0840-005\ [redacted] study # 707-007)***

5. A developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of PMEA administered intravenously to presumed pregnant rats, Lot # 393E93-01, [redacted] November 1, 1994, (94-TOX-0393-001, [redacted] 707-002)*

Review of reproductive and developmental toxicology studies:

1. Oral (gavage) fertility and general reproduction toxicity study of adefovir dipivoxil in rats, Lot # TX-840-96-03B, [redacted] May 16, 1997, (96-TOX-0840-001)

Groups of male and female [Crl:CD BR VAF/Plus (Sprague-Dawley); 25/sex/group)] rats were administered adefovir dipivoxil via gavage at dose levels of 0 (vehicle control), 1.2 (low), 6 (mid) or 30 mg/kg/day for females beginning 15 days before a 21-day cohabitation period and continuing through day 7 of presumed gestation or the day before scheduled sacrifice and, for males beginning 28 days before the cohabitation period and continuing until sacrifice. Male rats were sacrificed on study days 70 though 73 and presumed pregnant female rats were sacrificed on gestation day 20. Results-male rats: one rat (high) was found dead on day 49; the death was considered to be drug-related. Body weights: the high dosage group had significant reduced ($p<0.01$) body weight gains for the first 3 weeks and tended to have reduced body weight gains for the remainder of the period. Feed consumption: the absolute (g/day) feed consumption value (high) was significantly reduced ($p<0.5$ to 0.01) for the pre-cohabitation period and remained reduced thereafter. Fertility parameters: were unaffected. Every male rat mated: the mating resulted in pregnancy incidences ranging from 88% to 92%. Gross pathology: two male rats (high) had large pale kidneys; these lesions were considered to be drug-related. The absolute kidney weights of the left kidneys tended to be increased and the ratio of the right and left kidney weights to terminal body weights were significantly increased ($p<0.01$) in these two rats. Histopathology: indicated that a cortical tubular degeneration was present in 1/25 (mid; mild severity) and in 25/25 (high; mild-to-marked severity). Intestinal inflammation was present in 15/25 (high) rats. Female rats: all female rats survived to scheduled sacrifice. Body weights: gains for the two-week precohabitation period (days 1 to 15) were slightly reduced (high). Body weight gain was significantly reduced ($p<0.01$) during the gestation period (high). Absolute (g/day) and relative (g/kg/day) feed consumption values were slightly reduced (high) during the pre-cohabitation period. These parameters were significantly reduced ($p<0.05$-0.01) in the high dose group on gestation days 0-8 and unaffected thereafter. Estrous cycling,

mating and fertility parameters were unaffected. No gross lesions were attributed to the test article. Histopathology: evaluation of kidneys indicated that a cortical tubular degeneration was present in 9/25 rats (high; mild severity). Caesarean-sectioning or litter observations (litter averages for corpora lutea, implantations, live litter sizes, resorptions, percent resorbed conceptuses, fetal body weights and fetal sex ratios or the numbers of dams with any resorption) were not affected by the treatment. No gross external fetal alterations were attributable to drug.

Comments: The NOELs for systemic toxicity in this study was 1.2 mg/kg/day for male and 6 mg/kg/day for female rats. Based on a body surface area conversion factor, equivalent doses in humans would be 0.19 mg/kg/day for males and 0.97 mg/kg/day for females. Based on these data, the NOEL for effects of adefovir dipivoxil on fertility and reproductive performance in male and female rats is 30 mg/kg/day. Based on a body surface area conversion factor, equivalent dose in humans would be 4.87 mg/kg/day.

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

This study consisted of eight groups of presumed-pregnant female rats (Crl:CD BR VAF/Plus) and two groups of presumed-pregnant female New Zealand White rabbits receiving iv adefovir or oral gavage adefovir dipivoxil according to a study design shown in Table 6. Blood samples were collected at protocol specified timepoints and were analyzed by a validated [redacted] method.

Table 6
Study design

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

8	16	0		25	once daily for 10 days; days 6-15 of gestation	day 10
9	0	3		20	once on day 6 of gestation	day 1
10	0	3		20	once daily for 13 days; days 6-18 of gestation	day 13

Results: administration of adefovir (2.5 and 10 mg/kg/day) and adefovir dipivoxil (6.25 and 25 mg/kg/day) by iv injection or oral gavage, respectively, for up to ten consecutive days during the period of organogenesis was adequately tolerated in presumed pregnant rats. Likewise, oral gavage administration of adefovir dipivoxil (20 mg/kg/day) was adequately tolerated following up to 13 daily administrations to presumed pregnant rabbits during the period of organogenesis. This was supported by the absence of any test article-related abnormal observations or body weight changes during the study period. The observed pharmacokinetic parameters are shown in Table 7.

Table 7
Mean pharmacokinetic parameters of adefovir and dipivoxil adefovir in presumed pregnant female rats and rabbits

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

3. Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of adefovir dipivoxil in rats,

including a postnatal behavioral/functional evaluation, Lot # TX-840-97-01B, October 23, 1997, (97-TOX-0840-001)

Groups of presumed pregnant female [Crl:CD BR VAF/Plus (Sprague-Dawley); 25/sex/group)] rats were administered adefovir dipivoxil via gavage at dose levels of 0 (vehicle control), 2.5 (low), 10 (mid) or 40 mg/kg/day (high) beginning on gestation day (GD) 7 and continuing through gestation day 24 (rats that did not deliver a litter) or lactation day 20 (rats that delivered a litter). Results: all rats survived to the scheduled sacrifice. Body weight gains: were significantly reduced in the high dosage group ($p<0.01$) throughout the gestation dosage period (GDs 7-20) and the entire gestation period (GDs 0-20). Feed consumption: the absolute (g/day) and relative (g/kg/day) feed consumption values (high) were significantly reduced ($p<0.05$) on GDs 7-20. Natural delivery observations: the pregnancy rate, duration of gestation, total delivery time per litter, numbers of dams delivering litter, the litter averages for implantation sites per delivered litter and the gestation index (number of dams with live offspring/number of pregnant rats) were comparable among the four dosage groups and did not significantly differ. A significant decreased ($p<0.05$) number of dams (high) delivered on GD 22 and a significantly increased ($p<0.05$) number of dams (high) delivered on GD 23. These differences from the control group values were not considered related to the test article because duration of gestation and the total delivery times did not differ among the groups. F1 generation: average pup weights per litter were significantly ($p < 0.05$) decreased (high). All other litter observations were unaffected. There were no statistically significant or biologically important differences among the four dosage groups in the incidences of clinical or necropsy observations. The lower body weights for the high dose group rats (at weaning) as an indication of the toxicity, was evident throughout the observation period. However, body weight gains were not significantly different from the controls throughout the postweaning period. Feed consumption for the F1 generation was not adversely affected by the treatment. Sporadic incidences of statistically significant increases in relative feed consumption values (high) in male and female rats were reflective of the significantly reduced body weights at weaning. Sexual maturation (age at preputial separation or vaginal patency), postweaning behavior (passive avoidance and watermaze swim task), mating, fertility and Caesarean-sectioning observations (litter vital status and fetal gross observations) were comparable among the four groups. F2 generation: continued observations of the F1 generation from weaning through mating and Caesarean-delivery of an F2 generation revealed no adverse effects of the test article to the F0 dams. Treatment of the F1 generation rats did not affect litter averages for corpora lutea, implantations, live

litter sizes, resorptions, fetal body weight, fetal sex ratios and the numbers of dams with any resorptions.

Comments: Based on the results of this study, the NOELs for maternal and developmental, and reproductive toxicities were 10, and 40 mg/kg/day, respectively. Based on a body surface area conversion factor, equivalent doses in humans for maternal and developmental, and reproductive toxicities would be 1.62, and 6.5 mg/kg/day, respectively.

4. Oral (stomach tube) developmental toxicity study of bis-POMPMEA in Rabbits. Lot # TX840-95-05, March 27, 1996, (94-TOX-0840-005\ study # 707-007)

Groups of timed-pregnant female New Zealand White rabbits (20 animals/group) were administered orally (via stomach tube) bis-POMPMEA at dose levels of 0 (vehicle control), 1 (low), 5 (mid) or 20 mg/kg/day (high) once daily on day 6-18 of presumed gestation. Results: two deaths (control) and one (high) were attributed to intubation accidents. Average maternal body weight changes, body weights and absolute (g/day) and relative (g/kg/day) feed consumption values were unaffected; there were no statistically or biologically important differences among the four dosage groups. Caesarean-sectioning and litter observations were unaffected by the test article. The litter averages for corpora lutea, implantations, live and dead fetuses were comparable among the four dosage groups and did not differ significantly. No fetal external, soft tissue or skeletal malformations or variations were attributed to administration of the test article.

Comments: Toxicokinetic data were not provided. Based on the observation in this study, no maternal or fetal toxicity resulted from administration of bis-POMPMEA to pregnant New Zealand White rabbits. A dosage of 20 mg/kg/day may be considered the maternal and fetal NOELs. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 6.45 mg/kg/day.

5. A developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of PMEA administered intravenously to presumed pregnant rats, Lot # 393E93-01, November 1, 1994, (94-TOX-0393-001, 707-002)

Groups of presumed pregnant rats (strain: Crl:CD BR VAF/Plus; 25 rats/group) were administered PMEA intravenously once daily at dose levels of 0 (vehicle control), 2.5 (low), 10 (mid) or 20 mg/kg/day (high) during gestation days 6 through 15 to evaluate developmental toxicity and teratogenic potential of the test

compound. Results: three animals (1 vehicle control and 2 high) died as a result of errors in the injection procedure. Average maternal body weight gains were significantly reduced (P=0.05 to P=0.01) in mid and high dose groups for the entire injection period. Absolute (g/day) and relative (g/kg/day) feed consumption values were reduced (P=0.01) in both mid and high dose groups for the entire dosage period. Live fetal weights were significantly reduced (P=0.01) in mid and high dose groups and in male (P=0.5) fetuses (low). Fetal ossification of multiple sites at statistically significant incidences (P=0.05 to P=0.01) occurred (high) and a minimal delay in ossification occurred in the mid dose group. Analysis of fetal ossification site averages revealed significant reductions (P=0.05 to P=0.01) in the average number of ossified metacarpal bones (mid and high). A sporadic increase in the incidences of various malformations and in the incidence of supernumerary thoracic ribs (with associated increases and decreases in thoracic and lumbar vertebrae, respectively) also occurred in the high dose group. Fetal malformations (high) included depressed eye bulge (with associated small eye socket), anasarca (edematous body), gastroschisis, umbilical hernia, absent forepaw digit (with associated reductions in the number of metacarpals and phalanges), kinked tail, thoracic hemivertebra, unilateral ossification of the centrum in a thoracic vertebra (P=0.01), fused thoracic vertebral arches, fused ribs and a segmented rib. Significant litter and/or fetal incidences of a cervical rib at the 7th cervical vertebra (P=0.01, mid) and incompletely ossified pubes (P=0.01, low) were noted.

Comments: Treatment-related embryotoxicity and fetal malformations were observed at PMEA dosages of > 10 mg/kg/day (mid and high). Fetal malformation (incompletely ossified pubes) also occurred in low dose animals. Treatment-associated embryotoxicity and teratogenicity were observed at PMEA dosages of > 10 mg/kg/day but the dosages also resulted in significant maternal toxicity. Therefore, based on the results of this study, PMEA can not be categorized unequivocally as a teratogen because maternal toxicity was also seen at the same dose levels. The developmental NOEL for PMEA is 10 mg/kg/day since there were effects on live fetal weights at the low dose males.

VIII. SPECIAL TOXICOLOGY STUDIES:

Special toxicology studies summary:

1. **A dermal sensitization study in guinea pigs with bis-POM PMEA, Lot # 2166-C-1P, [redacted] April 18, 1997, (95-TOX-004/3337.5)***

2. **A primary skin irritation study in rabbits with bis-POM PMEA, Lot # 2166-C-1P, [redacted] November 16, 1995, (95-TOX-002/3337.4)***

3. **A primary eye irritation study in rabbits with bis-POM PMEA, Lot # 2166-C-1P, [redacted] April 21, 1997, (95-TOX-003/3337.3)***

Review of special toxicology studies:

1. A dermal sensitization study in guinea pigs with bis-POM PMEA, Lot # 2166-C-1P, [redacted] April 18, 1997, (95-TOX-004/3337.5)

Groups of male and female guinea pigs (5 animals/sex/group) were topically treated with 0 (challenge control) or 100% bis-POM PMEA (challenge treated), once weekly for 3 consecutive weeks. Following a 2-week rest period, a challenge test was performed whereby 100% bis-POM PMEA was topically applied to both challenge controls and challenge treated animals. Challenge responses of the treated were compared to those of the challenge controls. A positive control group (DNCB) was also included. Results: following challenge with 100% bis-POM PMEA, dermal scores of 0 to ± were noted in all test and challenge control animals. Group mean dermal scores were noted to be similar in the test animals as compared to the challenge control animals. Conclusions: based on the results of this study, bis-POM PMEA is not considered to be a contact sensitizer in guinea pigs.

2. A primary skin irritation study in rabbits with bis-POM PMEA, Lot # 2166-C-1P, [redacted] November 16, 1995, (95-TOX-002/3337.4)

Five male and one female New Zealand White rabbits were applied a 1" * 1" square 4 ply gauze patch containing 0.5 mg of bis-POM PMEA on the dorsal area of trunk. Care was taken to avoid abrading the skin during the clipping procedure. Removal and ingestion of drug was prevented by placing an elastic wrap over the trunk and test area. After a 4-hr exposure period, the elastic wrap and gauze patch were removed, and the animals were examined for signs of erythema and edema. The responses were scored at approximately 1, 24, 48 and 72 hr post treatment according to a Dermal Grading System. Results: exposure to bis-POM PMEA produced very slight erythema on 5/6 test sites at the 1 hr scoring interval. The dermal irritation resolved completely in all animals by the 48 hr scoring interval. Conclusion: under the conditions of this study, bis-POM PMEA was considered to be a slight irritant to the skin of the rabbit.

3. A primary eye irritation study in rabbits with bis-POM PMEA, Lot # 2166-C-1P, [redacted] April 21, 1997, (95-TOX-003/3337.3)

Groups of male and female New Zealand White rabbits (3/group) received a single 0.047 g (0.1 ml) dose of bis-POM PMEA in the conjunctival sac of the right eye. The contralateral eye remained untreated to serve as a vehicle control. No rinsing procedure of the eyes was utilized with the Group 1 (1 ♂ + 2 ♀); for Group 2 (2 ♂ + 1♀) rabbits, both eyes were rinsed with physiological saline at 30 seconds post-instillation of the test article. Test and control eyes were examined for signs of irritation for up to 28 days following dosing. Results-Group 1 (no rinsing): bis-POM PMEA produced corneal opacity in 3/3 test eyes by the 24 hr scoring interval which was confirmed by positive fluorescein dye retention. The corneal opacity resolved in 2/3 test eyes by study termination (day 28). Iritis was observed in 3/3 test eyes at the 1 hr scoring interval and resolved completely in all animals by the day 14 scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1 hr scoring interval. The conjunctival irritation resolved completely in all animals by study day 28. Additional ocular findings included sloughing of the corneal epithelium (1/3) and corneal neovascularization (3/3). Group 2 (rinsed eyes): bis-POM PMEA produced corneal opacity in 1/3 test eyes at the 24 hr scoring interval. The corneal opacity resolved by the 48 hr scoring interval. Iritis was observed in 3/3 test eyes at the 1 hr scoring interval but resolved completely in all animals by the 72 hr scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1 hr scoring interval. The conjunctival irritation resolved completely in all eyes by study day 7. Conclusions: based on these data, bis-POM PMEA is considered to be a severe irritant to the ocular tissue of the rabbit without a post-dose saline eye rinse (Group 1). Bis-POM PMEA should be considered a mild irritant to the ocular tissue of the rabbit following a 30 second post-instillation saline rinse (Group 2).

VIII: CONCLUSIONS AND RECOMMENDATIONS:

In the clinic, the test compound is being administered as an oral formulation (10 mg tablet). The human $AUC_{0-\infty}$ (at steady state) at the dose of 10 mg/day is 0.210 µg*hr/ml. The kinetic data from subchronic/chronic toxicity studies in animals showed that the mean AUC value at the therapeutic dose was found to be either equal or lower than that achieved in the nonclinical toxicity studies at the NOELs/NOAELs. Based on either the body surface area equivalence factors or drug exposure (AUC values), the dosages used in the clinic are lower than the NOELs/NOAELs

identified in animal studies (Appendix # 2).

Adefovir dipivoxil can be classified as Pregnancy Category C. Adefovir dipivoxil should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Treatment with adefovir dipivoxil at > 1 mg/kg/day for 13 or 52 weeks in cynomolgus monkeys caused decreased total and free carnitine levels; partial resolution of the carnitine deficits occurred in the treated animals relative to the controls after 4-weeks of recovery period.

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA. The sponsor submitted protocols, which have been approved by the Executive CAC, to initiate the two-year carcinogenicity studies in mice and rats. The carcinogenic potential of long-term administration of adefovir dipivoxil in mice was determined and the test article was not found to be oncogenic in mice.

The issue of labeling will be addressed when the NDA is submitted and the review of the labeling will be carried out separately.

IX: APPENDIX

1. Synopsis of adefovir dipivoxil and adefovir acute animal toxicity studies.

Table 1
Summary of adefovir dipivoxil and adefovir acute toxicity studies

Species	Dose level (mg/kg)	Approximate $LD_{..}$ (mg/kg)	Deaths	Approx. NOAEL (mg/kg)	BSA equiv dose in man (mg/kg)	Major toxic signs

adefovir dipivoxil Rats, po,	24 75 225	>225	0	75	12.17	Necrosis in GI tract
Mice, iv, adefovir	250 500	>500	0	250	20.27	Decreased activity, hunched bodies
Rats,iv, adefovir	100 50 500 1000	500	yes	100	16.23	Decreased activity, ataxia and lethargy

BSA = Body surface area equivalent factor

Table 2
Summary of adefovir acute toxicity studies

Species	Dose level (mg/kg)	Approximate LD, (mg/kg)	Deaths	Approx. NOAEL (mg/kg)	BSA equiv dose in man (mg/kg)	Major toxic signs
Rabbits, im, sc	25 50 75	>75	0	not identified	-	Local irritant at all dose levels
Monkeys, iv	150 500	>150	yes	not identified	-	Emesis, hypoactive, anorexia, multifocal hemorrhages in kidney

BSA = Body surface area equivalent factor

2. Synopsis of adefovir dipivoxil animal subchronic/chronic oral toxicity studies in animals and comparison with the clinical doses.

Table 1
Summary of adefovir dipivoxil subchronic/chronic oral toxicity studies in animals and comparison with the clinical dose (10 mg tablet, AUC = 0.210 µg*hr/ml)

Study	Dose levels (mg/kg/day	Major toxic signs, histopath. & laboratory	NOEL/NOAEL(mg/kg/day) & AUC (µg*hr/ml)	Safety margin relative to human

		findings		AUC
Mice 1-month (gavage)	5 20 80	Individual cecal gland cell necrosis at >5 mg/kg/day; ALT and WBC increased at 80 mg/kg/day	5 AUC: na	2.4 X (based on BSA)
Mice 13-week (gavage)	10 30 100	Hepatocellular karyo/cytomegaly, single cell necrosis, oval cell hyperplasia, and spleen/thymus lymphoid necrosis/atrophy	10 AUC = 1.61	7.6 X
Rat 2-week (gavage)	12 37	Increased kidney weights; renal tubular cell karyomegaly, renal tubule dilatation	12 AUC = 1.83	8.7 X
Rat 4-week (gavage)	4 12 37	Renal tubular megalocytosis, degeneration, lymphohistiocytic infiltration	4 AUC = na	
Rats 6-month (gavage)	0.4 2 10	Renal tubular cell karyomegaly	2 mg/kg/day AUC = 1.68	
Monkeys 1-month (gavage)	8 25 75	Decrease in body weight & food consumption; renal tubular megalocytosis; inflammatory changes in gastric epithelium; ALT, AST and CK increases	8 AUC = na	16 X (based on BSA)
Monkeys 3-month (gavage)	1 5 25	Renal tubular cell karyomegaly, individual cell necrosis; ALT, AST and CK increases; carnitine decreases	1 AUC = 0.25	1.2 X
Monkeys 12-month (gavage)	0.2 1 5	Renal tubular cell karyomegaly; increased ALT, CK and decreased carnitine	1 AUC = 0.25	1.2 X

Table 2

Relative target organ toxicity of adefovir dipivoxil in animal models

Species dose (mg/kg/day)	Dose level at which organ toxicity was observed; AUC (µg*hr/ml)				
	Kidney toxicity	GI toxicity	Liver toxicity	Lymphoproliferative tissue	CK elevation
Mice 10,30,100	>30 mg/kg/day AUC=7.37	none	>10 mg/kg/day AUC=1.61	>10 mg/kg/day AUC=1.61	not elevated
Rats 0.4,2,10	>10 mg/kg/day	none	none	none	none
Monkeys 1,5,25	>5 mg/kg/day	>1 mg/kg/day AUC=0.25	>5 mg/kg/day AUC=1.4	none	>5 mg/kg/day AUC=1.4
Sensitivity	rat>monkey>mice	monkey>rat>mice	monkey=mice>rat	mice>rat,monkey	monkey>rat

3. Synopsis of single oral dose pharmacokinetic parameters of adefovir dipivoxil in animals

Table 1
Pharmacokinetic parameters of adefovir dipivoxil following oral administration of single doses in animals

Species	Dose (mg/kg)	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)	Cl/F l/hr/kg	F (%)
Mouse	10	1.55	0.31	0.5	3.23	3.52	43.4
	30	4.33	0.85	1	3.73	3.78	40.5
	100	16.3	3.24	0.5	4.53	3.34	45.8
Rat	2	nd	0.08	1	nd	nd	nd
	4	nd	-	nd	nd	nd	nd

	10	1.51	0.28	1	6.65	3.61	28
	37	6.32	0.99	2	4	3.19	31.8
Woodchuck	15	2	0.32	4	-	4.2	11.3
Dog	21.4	12.7	1.5	1.9	7.3	0.92	18
	28.2	19.7	2.6	2.1	7.3	0.78	19
Monkeys	1	nd	0.04	1.13	-	nd	nd
	5	1.8	0.21	1.13	-	1.5	40.4
	20	2.2	0.61	0.9	-	5.45	27.3
	75	12.5	3.35	2	2.2	3.27	18.8

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4. Synopsis of multiple oral dose pharmacokinetic parameters of adefovir dipivoxil in animals

Table 1

Effect of repeated dosing of adefovir dipivoxil on the pharmacokinetics of adefovir dipivoxil in animal toxicology studies

Species	Dose (mg/kg/day) & Week	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (hr)	T½ (hr)	Cl/F l/hr/kg	F (%)
Mouse	10, WK 1	1.55	0.31	0.5	3.23	3.52	43..4
	WK 13	1.61	0.3	1	7.24	3.35	-
	30, WK 1	4.33	0.85	1	3.73	3.78	40.5
	WK 13	7.37	1.39	0.5	5.69	2.21	-
	100,WK 1	16.3	3.28	0.5	4.53	3.34	45.8
	WK 13	24.1	6.32	0.5	4.58	2.26	-
Rat	2, WK 1	-	0.08	1	-	-	-

	WK 26	-	0.13	2	-	-	-
	10, ~~WK~~ 1	1.51	0.28	1	6.65	3.61	28
	WK 26	1.68	0.45	1	3.45	3.21	31.2
Monkey	5, WK 1	1.8	0.21	1.13	9.82	1.5	40.4
	WK 12	1.22	0.19	1.38	7.17	2.21	27.4
	25 WK 1	5.54	1.88	0.81	5.3	2.25	24.7
	WK 12	6.35	1.59	0.75	5.1	2.14	28.3

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5. Synopsis of serum carnitine levels in monkeys administered adefovir dipivoxil for 13 and 52 weeks in toxicology studies

Table 1

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

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

Table 2

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

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

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