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APPROVAL PACKAGE FOR:

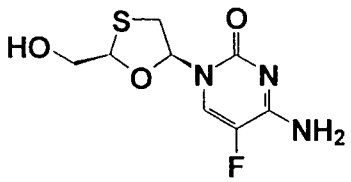
APPLICATION NUMBER

21-500

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-500



Review number: 000

Sequence number/date/type of submission: 000/September 03, 2002/original application

Information to sponsor: No

Sponsor: Triangle Pharmaceuticals, Inc.
4611 University Drive
Durham, NC 27707

Manufacturers for drug substance:

Reviewer name: Pritam S. Verma, Ph.D.

Division name: DAVDP
HFD-530

Review completion date: October 21, 2002

Drug:

Trade name: Coviracil® (200 mg Capsules and oral solution, 10 mg/ml)

Generic name: Emtricitabine

Code name: 524W91, FTC

Chemical Names: 4-Amino-5-fluoro-1-(2R-hydroxymethyl-[1,3]oxathiolan-5S-yl)-(1H)-pyrimidin-2-one

CAS registry number: 143491-57-0

Molecular formula/molecular weight: $C_8H_{10}FN_3O_3S$/247.24

Relevant INDs/NDAs/DMFs: [redacted]

Drug class: Nucleoside reverse transcriptase inhibitor (NRTI)

Indication: Treatment of HIV-1 infection

Clinical formulations: Composition of coviracil 200 mg capsules: The capsule consists of coviracil 200 mg, microcrystalline cellulose, NF — mg), crospovidone, NF (—mg), providone, USP (— mg) and magnesium stearate, NF — mg).

Route of administration: Oral

Proposed use: In combination with other antiretroviral agents for the treatment of HIV-1 infection

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: Adequate information

B. Recommendation for Nonclinical Studies: Adequate

C. Recommendation on Labeling: labeling issues will be dealt with separately

II. Summary of Nonclinical Findings

Pharmacology studies

Cytotoxicity of 524W91 and AZT: Human in vitro bone marrow progenitor colony forming assays were performed because there appeared to a correlation between toxicity to bone marrow progenitor cells in vitro and bone marrow suppression in vivo. In conclusion, the results indicated that in two different cell populations, 524W91 was relatively less toxic to the cells than AZT.

Proposed mechanism of action of 524W91: 524W91 is phosphorylated to the 5'-monophosphate by cellular deoxycytidine kinase, to the 5'-diphosphate by deoxycytidine monophosphate kinase, and then to the 5'-triphosphate by nucleoside diphosphate kinase. The 5'-triphosphate of 524W91 competitively inhibits the incorporation of 2'-deoxycytidine 5'-triphosphate into the homopolymeric template primer rI·dC catalyzed by RT. Because the 5'-triphosphate of 524W91 does not contain a 3'-hydroxyl group, its incorporation into nascent viral DNA results in chain termination.

Metabolism of 524W91 in human cells: The metabolism of 524W91 was studied in human hepatocellular carcinoma cells and CEM human T-lymphoblasts. Both cell lines anabolize 524W91 to 524W91 5'-triphosphate. The intracellular half-life of the triphosphate in these cells ranged from 2-5 hr. Besides mono, di and triphosphate of 524W91, two other metabolites (524W91-diphosphoethanolamine and 524W91-diphosphocholine) were identified in these studies, although at much lower concentrations.

Effects of 524W91 on human DNA polymerases: The 5'-triphosphate of 524W91 inhibited various human DNA polymerases. The K_is for the polymerases were as follows: α = 6.0 µM; ß = 17 µM; γ = 6 µM; and ε = 150 µM. The K_i for HIV-1 RT was 0.17 µM. In conclusion, 524W91 is a week inhibitor of the human DNA polymerases.

Effect of 524W91 on mitochondrial DNA synthesis in Molt-4 cells: Inhibition of mitochondrial DNA synthesis was assessed in an in vitro cell culture assay (Molt-4 cells) in which the ratio of mitochondrial to cellular DNA was determined after prolonged exposure of the cells to clinically relevant concentrations of the drug. 524W91 did not reduce the ratio of mitochondrial to cellular DNA up to a concentration of 100 µM after 7 days of continuous cell exposure. At 0.5 µM concentration of ddC, there was a 92% reduction in the ratio of mitochondrial DNA to cellular DNA.

Safety Pharmacology Studies:

General or Systemic Pharmacodynamic Effects (mice and rats): In a single dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. FTC did not affect behavior at any dose. In a subsequent single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, FTC did not affect body weight, rectal temperature or behavior at any dose. Conclusions: in mice, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day. In a single dose study, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 250, 500 or 1000 mg/kg, FTC did not affect body weight, rectal temperature or behavior at any dose. In a separate single dose study, male Wistar rats (5/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. FTC did not affect rectal temperature at any dose. Conclusions: in rats, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 163.13 mg/kg or 9.7 g/day for a 60 kg person.

Nervous System Effects: In an vitro experiment, 524W91 at 10 µM had no pharmacologically significant binding affinity at the adenosine (A_1 and A_2), adrenergic ($alpha_1$, $alpha_2$ and beta), angiotensin II, benzodiazepine, calcium channel (dihydropyridine and phenylalkylamine), cholinergic (M_1 and M_2), $dopamine_2$, $GABA_a$ gated chloride channel (TBPS), serotonergic ($5HT_{1A}$ and $5HT_2$) receptors. In an ex vivo serotonin response study, gastric fundus strips from male CD (SD) rats were incubated with FTC at 2.47 or 24.7 µg/ml concentrations. Contractile responses to 5-hydroxytryptamine (5-HT were measured before and after FTC exposure. Conclusions: exposure to FTC did not affect 5-HT induced gastric contraction at any concentration. In an ex vivo cholinergic response study, ilea from male albino Hartley guinea pigs were incubated with FTC at 2.47 or 24.7 µg/ml concentrations. Contractile responses to acetylcholine were measured before and after FTC exposure. Conclusions: exposure to FTC did not affect acetylcholine induced ilea contraction at either concentration. In a second study, ilea from 6 Hartley male guinea pigs were incubated with FTC at 2.47, 7.41 or 24.7 µg/ml concentrations. Contractile responses to FTC alone and interactions with acetylcholine induced contraction were measured. Conclusions: exposure to FTC did not induce ilea contraction or alter acetylcholine induced ileal contraction at any concentration. In a single dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, FTC did not affect neurological and autonomic at any dose. In a subsequent

single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, FTC did not affect reflexes at any dose. Conclusions: in mice, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day. In a single dose spontaneous locomotor activity study, male ICR mice (8/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. FTC did not affect spontaneous locomotion at any dose. In a separate single dose motor coordination (rotorod) study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. FTC did not affect motor coordination at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day. In a single dose hexobarbital potentiation (sleeping time) study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, FTC did not affect duration of anesthesia at any dose. In a separate single dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, FTC did not affect mortality and had no anticonvulsant activity at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day. In a single dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. FTC did not have analgesic activity at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day. In a subsequent single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, FTC had no analgesic activity at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day.
In a single dose rat study, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 250, 500 or 1000 mg/kg, FTC had no analgesic activity and did not affect reflexes at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 162.33 mg/kg or 9.7 g/day.
In a single dose conditioned avoidance response study, female Long-Evans rats (6/dose) were given FTC intra peritoneally at dose levels of 0, 30 or 100 mg/kg, FTC did not affect conditioned avoidance response at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 16.2 mg/kg or 974 mg/day.

Cardiovascular Effects: In a single dose rat study, conscious male Wistar rats (5/dose) were given FTC orally at dose levels of

0, 5, 10, 50 or 250 mg/kg. FTC did not affect heart rate or blood pressure at any dose. Conclusions: a single dose of 250 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 40.58 mg/kg or 2.4 g/day. In a single dose dog cardiovascular and respiratory effects study, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10 and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, FTC did not affect cardiovascular function parameters or heart rate or blood pressure response stimuli at any dose or time point. Conclusions: a dose level of 38.5 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 20.8 mg/kg or 1.2 g/day.

Respiratory Effects: In a single dose study in mice, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 750 or 1000 mg/kg, FTC did not affect respiratory rate at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day. In a single dose study in rats, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 100, 250, 750 or 1000 mg/kg, FTC did not affect respiratory rate at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 162.33 mg/kg or 9.7 g/day. In a single dose cardiovascular and respiratory effects study in dogs, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10 and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, FTC did not affect respiratory function parameters at any dose or time point. Conclusions: a dose level of 38.5 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 20.8 mg/kg or 1.2 g/day.

Renal effects: In a single dose renal function study in rats, male Long-Evans derived rats (6/dose) were given FTC orally at dose levels of 0, 30 or 100 mg/kg, FTC did not affect urine output, pH or electrolyte excretion at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 16.2 mg/kg or 974 mg/day.

Gastrointestinal Effects: In a single dose gastrointestinal motility study in rats, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, FTC did not affect gastrointestinal motility at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day.

Coagulation System Effects: In an in vitro receptor binding assay, the ability of FTC at 2.47 µg/ml to inhibit binding of radiolabeled platelet activating factor (PAF) to PAF receptors was evaluated using platelets for male New Zealand White rabbits.

FTC had essentially no effect on binding to the PAF receptor.

Pharmacokinetics/Toxicokinetics Studies:

Pharmacokinetics of FTC in Male Mice Following Single Oral and Intravenous Administration: Two groups of male CD-1 mice were given a single dose of 600 mg/kg FTC by either oral gavage or iv routes. After the iv administration, total body clearance was 1.3 L/kg/hr. Plasma clearance was described by a tri-exponential equation, with a $t_{½}$ of 4.14 hr. The volume of distribution at steady state was 1.1 L/kg. After the oral administration, absorption was rapid, with a T_{max} at 40 min and C_{max} at 139 µg/ml. The absolute bioavailability was 63%.

Pharmacokinetics of 524W91 in Male Mice Following Oral and Intravenous Administration: Two groups of male CD-1 mice were administered a single 10 mg/kg dose of a solution of 524W91 by either oral gavage or iv routes. After the iv administration, total body clearance was 2.33 L/kg/hr. Plasma clearance was bi-exponential, with $t_{½}\alpha$ = 4 min and $t_{½}ß$ = 23 min. The volume of distribution at steady state was 0.89 L/kg. After the oral administration, absorption was rapid, with T_{max} at 25.4 min and the C_{max} at 9.8 µM. The absolute oral bioavailability was 96%.

Pharmacokinetics of 100 mg/kg Oral and IV 524W91 in Male Mice: Two groups of male CD-1 mice were given a single dose of 100 mg/kg 524W91 by either oral gavage or iv routes. Blood samples were collected at various times up to 24 hr post-dose (5 mice per time point). After the iv administration, total body clearance was 2.23 L/kg/hr. Plasma clearance was described by a tri-exponential equation, with a $t_{½}\alpha$ of 1.7 min, a $t_{½}ß$ of 15.5 min and a $t_{½}\gamma$ of 82 min. The volume of distribution at steady state was 0.94 L/kg. After the oral administration, absorption was rapid, with a T_{max} at 24.5 min and C_{max} at 89 µM. The absolute bioavailability was 79%.

Pharmacokinetics of FTC in Male Cynomolgus Monkeys Following Single Oral and Intravenous Administration of FTC: Four male cynomolgus monkeys were administered a single 80 mg/kg dose of FTC by intravenous infusion and nasogastric gavage in a crossover design study. Select pharmacokinetic parameters were as follows: $AUCs_{0-\infty}$ (µg*hr/ml) were 86.1 ± 17.3 (iv) and 83.6 ± 11.2 (po). The absolute bioavailability was 97.4% in monkeys.

Pharmacokinetics of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration: Eight male cynomolgus monkeys were administered a single 10 mg/kg or 80 mg/kg dose of 524W91 (4 monkeys each dose) by intravenous infusion and nasogastric gavage in a crossover design study. Dose-independent kinetics was observed over the concentration range used in this study. The absolute bioavailability was 57.5% in this study.

Metabolic Disposition and Balance Studies in Male Mice Following Oral Administration of [6-^{3}H]-524W91 (120 mg/kg): Male CD-1 mice

(30-35 g) were dosed orally with 10 ml/kg of 12 mg/ml 524W91 containing 32 µCi/ml of [6-^{3}H]-524W91. In the feces, 18±3% of the dose was recovered, all as unchanged 524W91. In the urine, 64±7% of the radioactivity was recovered as unchanged 524W91, mostly in the 0-24 hr sample. The average cumulative recovery of radioactivity in the urine over the 72-hr period was 67±7% of the dose. Total recovery of radioactivity excreted in urine and feces was 85±4% of the dose. Three metabolites of 524W91 were measurable in the urine. These metabolites were tentatively identified as: 3742W92 (1.7%) and 3743W92 (2.0%), two isomeric, 3'-sulfoxides of 524W91 and 5-flurocytosine (1.4%). Traces of five other metabolites and a peak tentatively identified as tritiated water were also observed (<1%). The absolute oral bioavailability of 524W91 in the mouse has been shown to be 79% at 100 mg/kg. Metabolism of 524W91 appeared to be a minor pathway of clearance in the mouse.

FTC: [^{14}C]TP-0006: A tissue distribution and excretion study in rats: Twenty male Sprague Dawley nonpigmented rats (Group 1) and six male Long-Evans pigmented rats (Group 2) received a single 200 mg/kg oral dose containing approximately 135 µCi/kg of FTC: [^{14}C]TP-0006 via gavage. The distribution of radioactivity was rapid, with maximal levels occurring at 1 hr post dose in all non-GI tissues except inguinal lymph nodes (Cmax = 4 hr). Excluding the GI tract and urine, the highest concentrations of the radioactivity were found (in decreasing order) in the renal medulla, renal cortex, liver and urinary bladder. After reaching a Cmax, the concentration of radioactivity declined rapidly and by 24 hr post dose was non-detectable in all tissues except those of the GI tract. Distribution of radioactivity in pigmented rats was similar to nonpigmented rats, indicating that FTC did not bind to melanin to any significant extent. Recovery of total radioactivity in the urine and feces averaged 79.3% and 18.4% of the administered dose, respectively, with 95% of the total radioactivity excreted within 24 hr.

Metabolism and excretion of [^{14}C]-FTC following oral administration to male cynomolgus monkeys: Four adult male monkeys were given a single oral dose of 200 mg/kg FTC containing approximately 138 µCi of [^{14}C]-FTC (phase 1). After approximately 3 weeks, the four monkeys were dosed as described above (phase 2). For plasma, urine, feces and selected tissues analyzed, the parent drug was the predominant compound present. In urine, 29.2% of the dose was accounted for as FTC and 11.6% as metabolites, with M1, M2 and M3 together representing 8.4% of the dose. M1 and M2 are putative 3-sulfoxide diastereomers of FTC and M3 is a 2-O-glucuronide of FTC. In feces, 34.4% of the dose was accounted for as FTC and only 1.3% of the dose as metabolites, suggesting incomplete absorption or possible intestinal secretion of FTC in monkeys. Unchanged FTC represented the great majority of radioactivity present in urine (74%) and feces (97%), indicating

that FTC is not extensively metabolized.

Metabolic Disposition of 80 mg/kg Orally Administered [6-^{3}H]-524W91 in Cynomolgus Monkeys: The metabolism and elimination in urine and feces of 80 mg/kg orally administered [6-^{3}H]-524W91 containing 0.44 mCi/mmole was studied in four female cynomolgus monkeys. After 72 hr, 41% of the dose was excreted into the urine. Most of the urinary excretion occurred (33±9%) in the first 8 hr post-dose sample. Recovery of radioactivity in the feces averaged 33% of the dose after 72 hr. Recovery of radioactivity in the cage wash averaged 10% of dose over the same time period. The overall recovery of dose was 84%. Radiochemical-analysis of urine and fecal samples showed that unchanged 524W91 was 64% of the recovered dose in the urine and 98% of the feces. The major urinary metabolites constituted 11% of the dose. They were designated as: M200, M350, M950, M1030, M1500 and M1980. M350 co-eluted with 5-fluorocytosine, M950 and M1030 co-eluted with two isomeric 3'-sulfoxides of 524W91, and M1980 co-eluted with the deaminated metabolite of 524W91. Another potential urinary metabolite, 5-fluorouracil, was less than 0.02% of the dose. In the feces, unchanged 524W91 was the only quantifiable compound present. Traces of M950 and M1030 were detectable. Metabolism is a minor contributor to clearance of 524W91 in the monkey, since 64% of the radioactivity in the urine was present as parent compound. Approximately 11% of the dose was metabolized to a compound that co-eluted with a 3'-sulfoxide of 524W91.

Pharmacokinetics, excretion and tissue distribution of [^{14}C]-FTC following oral administration to male cynomolgus monkeys: Four adult male monkeys were given a single oral dose of 200 mg/kg FTC containing approximately 138 µCi of [^{14}C]-FTC (phase 1). After approximately 3 weeks, the four monkeys were dosed as described above (phase 2). After phase 1 administration, blood and plasma concentrations declined rapidly over time. The mean total recovery of radioactivity dose in urine, feces, cage wash and cage wipes over the 120 hr collection interval was 84.5%. Urine and feces accounted for a mean of 40.8% and 35.3% of the total radioactive dose, respectively, with 66.4% of the radioactivity excreted within 48 hr post dose. The cage wash and cage wipes accounted for an additional 8.31% of the total radioactive dose. At 1 hr following phase 2 dose administration, with the exception of the gastrointestinal tract tissues (small intestine, large intestine and stomach), the highest mean concentrations of radioactivity in the tissues were observed in the kidneys (596 µg/g) and liver (121 µg/g). The ratios for radioactivity concentrations found in the brain, cerebrospinal fluid, bone, aqueous humor and eyes as compared to blood were 0.028, 0.031, 0.039, 0.063 and 0.098, respectively indicating distribution of radioactivity into these tissues ranging from 2.17 to 7.53 µg/g.

Toxicokinetics report for a six month oral (gavage) toxicity

study in mice given FTC with 3-month interim kill: Groups of male and female CD-1 mice (10 animals/sex/group) were administered FTC via oral gavage at dose levels of 0 (0.5% methylcellulose, vehicle control) 167 (low), 500 (mid) or 1500 mg/kg/day (high). At 3 and 6 months of exposure, plasma samples were collected. FTC was rapidly absorbed with Cmax occurring by 30-60 min post dosing. The systemic exposure to FTC and the peak plasma concentrations increased linearly as the dose increased from 167 to 1500 mg/kg/day. FTC was eliminated from the plasma with T½ of 3-4 hr. Cmax and AUC estimates in both sex groups obtained at 6-month were higher than those obtained at 3-month, especially for the high dose level.

Protein Binding of 524W91 in Human, Monkey, Mouse and Rabbit Plasma: The binding of [^{3}H]-524W91 to human, monkey, mouse, and rabbit plasma proteins was determined over a concentration range of 0.02-200 µg/ml by equilibrium dialysis at 37°C. The mean percentage bound for all species studied was less than or equal to 3.6%, with no indication of concentration dependency. Because of the low extent of binding to human plasma at therapeutic concentrations, drug interactions mediated by protein binding displacement are not expected with 524W91.

General Toxicology Studies:

FTC: An acute iv and oral toxicity study in mice and acute oral toxicity study in rats: Groups of male and female mice and rats were administered FTC at dose levels of 700 (mouse iv), 1050 (mouse po) or 1400 (rat oral) mg/kg. After dosing, behavior changes of the animals were observed daily for 14 days. No animal died during the study. There were no signs of toxicity. There were no changes in body weights or feed consumption. The toxic or fatal dose of FTC following iv and oral administrations is greater than 700 and 1050 mg/kg, respectively. In rats, the oral toxic or fatal dose of FTC is greater than 700 mg/kg.

An Acute Oral Toxicity Study in Mice: A group of 5 male and 5 female CD-1 mice were fed via oral gavage a single 4 g/kg dose of 524W91 in 0.5% methylcellulose. There were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. The median lethal oral dose was greater than 4 g/kg.

An Acute Intravenous Toxicity Study in Mice: A group of 5 male and 5 female CD-1 mice were administered a single iv injection of 524W91 at a dose level of 200 mg/kg in 0.9% sterile saline. There were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. The median lethal iv dose was greater than 200 mg/kg.

An Acute Oral Toxicity Study in Rats: A group of 5 male and 5 female CD rats were fed via oral gavage a single 4 g/kg dose of 524W91 in 0.5% methylcellulose. There were no deaths; no clinical signs were noted throughout the study; and no treatment-related

gross findings were noted. The median lethal oral dose was greater than 4 g/kg.

Acute Intravenous Toxicity Study in Rats: A group of 5 male and 5 female CD rats were administered a single iv injection of 524W91 at a dose level of 200 mg/kg in 0.9% sterile saline. There was no vehicle control group in this study. There were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. The median lethal iv dose was greater than 200 mg/kg.

A 14-Day Oral Toxicity Study in Mice: Groups of male and female CD-1 mice were administered FTC via oral gavage once daily at dose levels of 0 (0.5% methylcellulose, vehicle control), 120 (low), 600 (mid) or 3000 mg/kg/day (high) for a period of 14 days. No adverse clinical findings were seen. A daily dose of 3000 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 243.3 mg/kg/day or 14.5 g/day for a 60 kg person.

A 14-Day Oral Bridging Toxicity Study in Mice: Two groups of male and female CD-1 mice were administered FTC via oral gavage twice a day approximately 6 hr apart at dose levels of 0 (0.5% methylcellulose, vehicle control) or 60 mg/kg/dose (the total dose of 120 mg/kg/day; the total dose volume of 20 ml/kg/day) for a period of 14 days. No adverse clinical findings were seen. A daily dose of 120 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 9.73 mg/kg/day or 584 mg/day for a 60 kg person.

A Thirty Day Oral Toxicity Study in Mice: Groups of male and female mice were administered 524W91 via oral gavage once daily at dose levels of 0 (0.5% methylcellulose, vehicle control), 120 (low), 600 (mid) or 3000 mg/kg/day (high) for a period of 32 days. No drug-related clinical signs were noted. The daily administration of 3000 mg/kg/day resulted in decreased RBC, Hb and HCT and increased MCV, MCH and RDW. A daily dose of 600 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 48.66 mg/kg/day or 2.9 g/day for a 60 kg person.

A one-month mouse oral toxicity study of FTC containing FTU and two potential process impurities: Groups of male and female CD-1 mice (10 animals/sex/group) were administered by gastric intubation, a suspension of FTC (containing 0.016% FTU, 0.00063% thioacetate and 0.012% disulfide) at dose levels of 0 (vehicle control), 133 (low), 400 (mid) or 1200 mg/kg/day (high) once daily for a month. There were no clinical signs noted during the treatment. Statistically significant heavier kidneys (both absolute and relative) were recorded for male mice (high). A daily dose of 400 mg/kg/day may be considered the NOAEL in this study. Based on a body surface area factor, an equivalent dose in humans would be 32.44 mg/kg/day or 1946 mg/day for a 60 kg person.

Six-month oral toxicity study (with a three month interim

sacrifice) in mice given 524W91: Groups of male and female CD-1 mice (25 animals/sex/group) received 524W91 via oral gavage at dosage levels of 0 (vehicle control), 120 (low), 600 (mid) or 3000 mg/kg/day (high) for a period of three months. Organ Weights: there was a reversible increase in absolute and relative thyroid weights in female mice (high). There were no histopathological changes to correlate with the finding. The daily administration of 3000 mg/kg/day of 524W91 for 6 months resulted in a reversible decrease in RBC and an increase in MCV, MCH and RDW. A dose of 600 mg/kg/day may be considered the NOEL. On the basis of the body surface area, an equivalent dose for humans would be 48.66 mg/kg/day or 2.9 g/day for a 60 kg person.

Six month oral toxicity study in mice given FTC with a 3-month interim kill: 3-month interim report: Four groups of male and female mice (30 animals/sex/group) were administered FTC via oral gavage once daily at dose levels of 0 (vehicle control), 167 (low), 500 (mid) or 1500 mg/kg/day (high) for a period of 13 weeks. There were no clinical observations considered to be due to FTC administration. Hematology: a small statistically significant ($p<0.05$) increase was seen for MCH and MCV in the high dose animals at 6 months. A daily dose of 1500 mg/kg/day may be considered the NOAEL in this study. Based on a body surface area factor, an equivalent dose in humans would be 121.65 mg/kg/day or 7299 mg/day for a 60 kg person.

A 3-month oral toxicity study for bioassay dose selection in CD rats: Four groups of male and female CD rats (28 animals/sex/group) were administered FTC via oral gavage (20 ml) once daily at dose levels of 0 (0.5% methylcellulose in water, vehicle control), 120 (low), 600 (mid) or 3000 mg/kg/day (high) for a period of 13 weeks. No drug related clinical findings were observed during the study. A daily dose of 3000 mg/kg/day may be considered the NOAEL in this study. Based on a body surface area factor, an equivalent dose in humans would be 487 mg/kg/day or 29.2 g/day for a 60 kg person.

A Thirty Day Oral Toxicity Study in Cynomolgus Monkeys: Groups of male and female cynomolgus monkeys (5 animals/sex/group) were administered 524W91 via oral gavage once daily at dose levels of 0 (0.5% methylcellulose, vehicle control), 80 (low), 400 (mid) or 2000 mg/kg/day (high) for a period of 28 days. Drug-related clinical signs included a higher incidence of soft feces in the high dose males were seen. A daily dose of 400 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 133 mg/kg/day or 7.9 g/day for a 60 kg person.

A 3-Month Oral Toxicity Study in Cynomolgus Monkeys Given 524W91: Groups of male and female cynomolgus monkeys (5 animals/sex/group) were administered 524W91 via oral gavage twice daily at dosage levels of 0 (0.5% methylcellulose, vehicle control), 40 (low), 200 (mid) or 1000 mg/kg/day (high) for a

period of 13 weeks. One male (high) was noted to have emesis immediately postdose during weeks 1 and 5. There were no changes in body weights. A daily dose of 200 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 66 mg/kg/day or 3.96 g/day for a 60 kg person.

FTC: 52-Week oral toxicity study in cynomolgus monkeys with a 4-week recovery period: Four groups of male and female cynomolgus monkeys (4-8 animals/sex/group) were administered FTC via oral gavage twice daily (5-hr apart) at total dose levels of 0 (vehicle control; 8 animals/sex), 50 (low), 200 (mid) or 500 mg/kg/day (high; 8 animals/sex) for a period of 52 weeks. there were no clinical observations considered to be due to FTC administration. There were no drug-related effects observed except a small (not statistically significant) decrease in mean erythrocyte counts and a significant increase in mean corpuscular hemoglobin values observed in female monkeys (high). FTC was rapidly and well absorbed following the oral administration. A daily dose of 200 mg/kg/day may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 66.66 mg/kg/day or 4 g/day for a 60 kg person.

Genetic Toxicology Studies:

Mutagenicity Test on FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay (Ames Test): FTC was tested for its ability to induce gene mutations in two versions of the Salmonella-E. coli/mammalian-microsome mutagenicity assay, using tester Salmonella strains TA98, TA100, TA1535 and TA1537, and E. coli strain WP2uvrA, both in the presence and in the absence of rat liver S9 metabolic activation. FTC exerted no detectable mutagenic activity.

In vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay): FTC was tested in the L51178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay. Exposures to FTC were in the presence and absence of Aroclor-induced rat liver S9 metabolic activation. FTC was found to be negative in the mouse lymphoma mutagenesis assay.

In vivo Mammalian Erythrocyte Micronucleus Test: Groups of male mice (5-15/group) were orally gavaged a single dose of FTC at dose levels of 0 (control), 500 (low), 1000 (mid) or 2000 mg/kg (high) to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. FTC did not induce a significant increase in micronucleated polychromatic erythrocytes in male mice. FTC was concluded to be negative in the micronucleus assay.

Mutagenicity Test on 524W91 in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test): 524W91 was tested for its ability to induce gene mutations in two versions of the Ames Salmonella/mammalian-microsome mutagenicity assay, using tester

strains TA1535, TA1537, TA1538 and TA100, both in the presence and in the absence of rat liver S9 metabolic activation. In the Ames assay, 524W91 exerted no detectable mutagenic activity.

Carcinogeniticy study protocols:

FTC mouse protocol: Two-year oral oncogenicity study in CD-1 mice: The treatment levels (0, 75, 250 or 750 mg/kg/day) were chosen based on a 6-month oral gavage toxicity study in mice. The low dose of 75 mg/kg/day is anticipated to give exposures approximately 3-fold higher than the expected human exposures based on linear extrapolation. The mid dose of 250 mg/kg/day is expected to give exposures approximately 10-fold higher than the human exposure, and was arithmetically derived to be mid way between the low (75 mg/kg/day) and high (750 mg/kg/day) doses. The protocol has been approved by the ECAC.

FTC rat protocol: Two-year oral oncogenicity study in CD rats: The treatment levels (0, 60, 200 or 600 mg/kg/day) were chosen based on a 90-day oral gavage toxicity study in rats. The sponsor has utilized the pharmacokinetics endpoint for the determination of the top dose that meets criteria in the ICH Guideline regarding acceptability of 25 times exposure as being adequate. Thus, a dose level of 600 mg/kg/day is expected to provide systemic exposures > 30 times human exposure in the case of both male (35.5 times) and female (39 times) rats. The mid and low doses were arithmetically determined, with the low dose expected to provide approximately 3 times human exposure. The protocol has been approved by the ECAC.

Reproductive and developmental toxicology studies:

FTC: Study of fertility and early embryonic development in CD-1 mice (Segment I): Groups of male and female mice (20-21 animals/sex/group) were administered FTC via oral gavage daily at dose levels of 0 (vehicle control), 250 (low), 500 (mid) or 1000 mg/kg/day (high). Male mice were dosed 28 days prior to breeding, 21 days during mating and 21 days post-mating. Females were dosed 14 days prior to breeding, 21 days during mating and 7 days during gestation. There was no evidence of F_0 male or female reproductive toxicity. The NOAEL was 1000 mg/kg/day for both adult male and female systemic toxicity under the conditions of the study. The NOAEL for reproductive and developmental toxicity was also 1000 mg/kg/day. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg/day or 4866.18 mg/day for a 60 kg person.

Study of fertility male rats (Segment I) given 524W91 by gavage: Groups of male CD rats (Sprague Dawley; 25 animals/group) were administered test compound via oral gavage daily at dose levels of 0 (vehicle control), 150 (low), 750 (mid) or 3000 mg/kg/day (high) for 73 days prior to mating and throughout the mating period, then euthanatized and necropsied. Naive, untreated female

rats were cohabited at a 1:1 ratio with treated males and monitored for evidence of mating. There were no adverse effects on male, fertility, reproductive performance, or sperm count or mortality. The NOAELs for general toxicity in male rats, and reproductive toxicity in male rats and early fetal development in naive females were 750 mg/kg/day and 3000 mg/kg/day, respectively. Based on a body surface area factor, an equivalent dose in humans would be 121.75 mg/kg/day or 7.3 g/day and 487 mg/kg/day or 29 g, respectively, for a 60 kg person. The ratio to the clinical dose was > 37-fold.

A study of the effects of FTC on embryo/fetal development in mice (Segment II): Groups of presumed pregnant female mice (25 dams/group) were administered FTC via oral gavage twice daily at total dose levels of 0 (vehicle control), 250 (low), 500 (mid) or 1000 mg/kg/day (high) during gestation days 6 through 15. A dose of 1000 mg/kg/day (administered twice daily 6-hr apart as 500 mg/kg/day) may be considered the NOAEL for maternal toxicity and developmental toxicity of FTC. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg/day or 4866.18 mg/day for a 60 kg female. The ratio to the clinical dose was > 24-fold.

Toxicokinetics study to determine fetal exposures in CD-1 mice given FTC orally (gavage): Eight pregnant CD-1 mice were administered FTC via oral gavage at dose levels of 0 (0.5% methylcellulose, vehicle control) or 1000 mg/kg/day. The mean maternal plasma concentration of FTC was 137.1 µg/ml. The mean concentration of FTC in pooled fetal homogenate was 55.7 µg/ml. The mean fetal/maternal concentration ratio was 0.41.

A study of the effects on embryo/fetal development in rabbits (Segment II): Groups of artificially inseminated New Zealand White rabbits (25 animals/group) were orally dosed (gavage) with FTC at dose levels of 0 (0.5% methylcellulose, 5 ml/kg/dose, vehicle control) 100 (low), 300 (mid) or 1000 mg/kg/day (high) twice daily from gestation days 7 through 19. No maternal toxicity was apparent at a dose level of 100 mg/kg/day. Body weight gain and food consumptions were reduced ($p<0.05$ or 0.01) in the mid (gestation days 15-20) and high (11-15) dosed animals. No developmental toxicity was observed at any dose level. A dose level of 100 mg/kg/day was considered to be the NOAEL for maternal toxicity and 1000 mg/kg/day was considered to be the NOAEL for developmental toxicity of FTC in rabbits. Based on the body surface area factor, an equivalent dose in humans would be 31.94 mg/kg/day or 1.9 g/day (NOAEL for maternal toxicity) and 319.5 mg/kg/day or 19.1 g/day (NOAEL developmental toxicity). The exposure ratios to the clinical dose were: 8.7 (NOAEL for maternal toxicity) and 125 (developmental toxicity).

FTC: Pre- and postnatal development in CD-1 mice (Segment III): Groups of presumed pregnant female mice (25 dams/group) were administered FTC via oral gavage daily at dose levels of 0 (vehicle control), 250 (low), 500 (mid) or 1000 mg/kg/day (high)

during gestation days 6 through 20. A dose of 1000 mg/kg/day may be considered the NOAEL for maternal toxicity of F_0 dams. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg/day or 4866.18 mg/day for a 60 kg female. The NOAEL for post-weanling F_1 male and female toxicity was 1000 mg/kg/day. The NOAEL for F_0 and F_1 reproductive toxicity and for F_2 developmental toxicity was 1000 mg/kg/day.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: _____

Concurrences:

HFD-530/JFarrelly
HFD-530/PVerma

Disk
HFD-530/JFarrelly

TABLE OF CONTENTS-PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY:

Coviracil (also known as FTC) is the brand name of emtricitabine, a nucleoside analogue reverse transcriptase inhibitor, which has been proposed for the treatment of HIV-1 infection. The sponsor suggested that the compound showed potent in vitro and in vivo antiretroviral activity against HIV and HBV. FTC is unique in that the enantiomorph that is the most active antiviral agent has a 1-ß-L configuration rather than the 1-ß-D configuration found in the natural nucleosides. Also, FTC has a unique oxathiolane

ring system.

FTC, a synthetic nucleoside analog of cytosine, is phosphorylated by cellular enzymes to form FTC-5-triphosphate which inhibits the activity of the HIV-1 reverse transcriptase through high affinity binding, competing with the natural substrate deoxycytidine 5-triphosphate. FTC-5-monophosphate is efficiently incorporated into nascent proviral chain DNA resulting in chain termination.

Preclinical studies to characterize the absorption, distribution, metabolism and excretion of FTC have been performed in mice, rats and monkeys. These studies demonstrate that FTC is rapidly absorbed after oral administration. FTC is widely distributed throughout the body, with a volume of distribution similar to that of total body water. FTC does not undergo extensive first-pass or systemic metabolism and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeded the glomerular filtration rate, suggesting the drug was actively secreted by renal tubules into the urine. FTC disposition was similar between the three animal species studied, and did not demonstrate significant dose-dependent kinetics.

The safety pharmacology, general systemic toxicology, genotoxicology and reproductive toxicology studies of FTC had been characterized in a variety of animal species. In the general toxicology studies, FTC was well tolerated for up to a year at doses producing systemic exposures much greater than those produced in patients at the recommended 200 mg ($AUCss_{0-24hr}$ = 1.0 µg*hr/ml) daily dose. Toxicity was limited to animals in the high dose group and to mild reversible anemia in mice (3000 mg/kg/day for 6 months)and soft feces in female monkeys (2000 mg/kg/day for 1 month). FTC was not genotoxic, did not adversely affect reproduction or embryo fetal development. Presently, the sponsor has submitted this application for review by the Division.

II. PHARMACOLOGY

Pharmacology Studies Summary: The following studies were provided as abstracts from published scientific literatures

1. **Cytotoxicity of 524W91 and AZT**
2. **Proposed mechanism of action of 524W91**
3. **Metabolism of 524W91 in human cells**
4. **Effects of 524W91 on human DNA polymerases**
5. **Effect of 524W91 on mitochondrial DNA synthesis in Molt-4 cells**

Review of Pharmacology Studies:

1. Cytotoxicity of 524W91 and AZT

Human in vitro bone marrow progenitor colony forming assays were performed because there appeared to a correlation between toxicity to bone marrow progenitor cells in vitro and bone marrow suppression in vivo. The results of 524W91 in vitro bone marrow toxicity in comparison with AZT are shown in Table 1. In conclusion, the results indicated that in two different cell populations, 524W91 was relatively less toxic to the cells than AZT.

Table 1
Bone Marrow Stem Cell Toxicity of 524W91 and AZT

Cell Population	524W91 IC_{50} (μM)	AZT IC_{50} (μM)
BFU-E	220±8 (n=6)	0.30±0.06 (n=55)
CFU-GM	300±40 (n=6)	10±3 (n=55)

2. Proposed mechanism of action of 524W91

524W91 is phosphorylated to the 5'-monophosphate by cellular deoxycytidine kinase, to the 5'-diphosphate by deoxycytidine monophosphate kinase, and then to the 5'-triphosphate by nucleoside diphosphate kinase. The 5'-triphosphate of 524W91 competitively inhibits the incorporation of 2'-deoxycytidine 5'-triphosphate into the homopolymeric template primer rI·dC catalyzed by RT. Because the 5'-triphosphate of 524W91 does not contain a 3'-hydroxyl group, its incorporation into nascent viral DNA results in the chain termination.

3. Metabolism of 524W91 in human cells

The metabolism of 524W91 was studied in human hepatocellular carcinoma cells and CEM human T-lymphoblasts. Both cell lines anabolize 524W91 to 524W91 5'-triphosphate. The intracellular half-life of the triphosphate in these cells ranged from 2-5 hr. Besides mono, di and triphosphate of 524W91, two other metabolites (524W91-diphosphoethanolamine and 524W91-diphosphocholine) were identified in these studies, although at much lower concentrations.

4. Effects of 524W91 on human DNA polymerases

The 5'-triphosphate of 524W91 inhibited various human DNA polymerases. The K_is for the polymerases were as follows: α = 6.0 μM; ß = 17 μM; γ = 6 μM; and ε = 150 μM. The K_i for HIV-1 RT was 0.17 μM. In conclusion, 524W91 is a week inhibitor of the human DNA polymerases.

5. Effect of 524W91 on mitochondrial DNA synthesis in Molt-4 cells

Inhibition of mitochondrial DNA synthesis was assessed in an in vitro cell culture assay (Molt-4 cells) in which the ratio of mitochondrial to cellular DNA was determined after prolonged exposure of the cells to clinically relevant concentrations of the drug. 524W91 did not reduce the ratio of mitochondrial to cellular DNA up to a concentration of 100 μM after 7 days of continuous cell exposure. At 0.5 μM concentration of ddC, there was a 92% reduction in the ratio of mitochondrial DNA to cellular DNA.

III. SAFETY PHARMACOLOGY

Safety Pharmacology Studies Summary: The following studies were provided as summary reports.

1. General or Systemic Pharmacodynamic Effects (mice and rats)
2. Nervous System Effects
3. Cardiovascular Effects
4. Respiratory Effects
5. Renal effects
6. Gastrointestinal Effects
7. Coagulation System Effects

Safety Pharmacology Studies Review:

1. General or Systemic Pharmacodynamic Effects (mice and rats)

Mice

In a single dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, then observed for 7 days to collect data on behavioral effects. FTC did not affect behavior at any dose. In a subsequent single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, then observed for 7 days to collect data on body weight, rectal temperature, and behavior. FTC did not affect body weight, rectal temperature or behavior at any dose. Conclusions: in mice, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day.

Rats

In a single dose study, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 250, 500 or 1000 mg/kg, then observed for 7 days to collect data on behavioral effects. FTC did not affect body weight, rectal temperature or behavior at any dose. In a separate single dose study, male Wistar rats (5/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. Rectal temperature was measured before dosing at intervals up to 2 hr postdose. FTC did not affect rectal temperature at any dose. Conclusions: in rats, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 163.13 mg/kg or 9.7 g/day for a 60 kg person.

2. Nervous System Effects

In vitro

524W91 at 10 µM had no pharmacologically significant binding affinity at the adenosine (A_1 and A_2), adrenergic (alpha$_1$, alpha$_2$ and beta), angiotensin II, benzodiazepine, calcium channel (dihydropyridine and phenylalkylamine), cholinergic (M_1 and M_2), dopamine$_2$, $GABA_a$ gated chloride channel (TBPS) or serotonergic ($5HT_{1A}$ and $5HT_2$) receptors.

Ex vivo

In an ex vivo serotonin response study, gastric fundus strips from male CD (SD) rats were incubated with FTC at 2.47 or 24.7 µg/ml concentrations. Contractile responses to 5-hydroxytryptamine (5-HT were measured before and after FTC exposure. Conclusions: exposure to FTC did not affect 5-HT induced gastric contraction at any concentration.

In an ex vivo cholinergic response study, ilea from male albino Hartley guinea pigs were incubated with FTC at 2.47 or 24.7 µg/ml concentrations. Contractile responses to acetylcholine were measured before and after FTC exposure. Conclusions: exposure to FTC did not affect acetylcholine induced ilea contraction at either concentration. In a second study, ilea from 6 Hartley male guinea pigs were incubated with FTC at 2.47, 7.41 or 24.7 µg/ml concentrations. Contractile responses to FTC alone and interactions with acetylcholine induced contraction were measured. Conclusions: exposure to FTC did not induce ilea contraction or alter acetylcholine induced ileal contraction at any concentration.

Mice

In a single dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, then observed for 7 days to collect data on neurological and autonomic effects. FTC did not affect neurological and autonomic receptors at any dose. In a subsequent single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, then observed for 7 days to collect data on reflexes. FTC did not affect reflexes at any dose. Conclusions: in mice, a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day.

In a single dose spontaneous locomotor activity study, male ICR mice (8/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. Spontaneous activity was recorded at 15 min interval for 2 hr postdose. FTC did not affect spontaneous locomotion at any dose. In a separate single dose motor coordination (rotorod) study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. Ability to remain on a rotating rod was evaluated at 0.5, 1 and 2 hr postdose. FTC did not affect motor coordination at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day.

In a single dose hexobarbital potentiation (sleeping time) study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, then anesthetized with hexobarbital 1 hr later. Time to recovery from anesthesia was measured. FTC did not affect duration of anesthesia at any dose. In a separate single dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, then given a maximal electrical shock at 1 hr postdose. The occurrence of death or tonic/clonic convulsions was recorded. FTC did not

affect mortality and had no anticonvulsant activity at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day.

In a single dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg. Time to tail flick response to an uncomfortable heat stimulus was recorded before an 1 hr after dosing. FTC did not have analgesic activity at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day.

In a subsequent single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 500, 750 or 1000 mg/kg, then evaluated for analgesia by the tale-flick test. FTC had no analgesic activity at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day.

Rats

In a single dose study, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 250, 500 or 1000 mg/kg, then evaluated for analgesia and reflexes. FTC had no analgesic activity and did not affect reflexes at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 162.33 mg/kg or 9.7 g/day.

In a single dose conditioned avoidance response study, trained ovariectomized female Long-Evans rats (6/dose) were given FTC intra peritoneally at dose levels of 0, 30 or 100 mg/kg, then tested for their ability to respond to an audio-visual cue to avoid foot-shock. FTC did not affect conditioned avoidance response at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 16.2 mg/kg or 974 mg/day.

3. Cardiovascular Effects

Rats:

In a single dose cardiovascular study, conscious male Wistar rats (5/dose) were given FTC orally at dose levels of 0, 5, 10, 50 or 250 mg/kg. Heart rate and arterial blood pressure were measured before dosing and at 5, 30 and 60 min postdose. FTC did not affect heart rate or blood pressure at any dose. Conclusions: a

single dose of 250 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 40.58 mg/kg or 2.4 g/day.

Dogs:

In a single dose cardiovascular and respiratory effects study, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10 and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more min. Heart rate, arterial blood pressure and lead II electrocardiogram were measured at intervals, and blood pressure responses to norepinephrine, acetylcholine, carotid artery occlusion and vagal nerve stimulation were evaluated 30 min after the last dose. FTC did not affect cardiovascular function parameters or heart rate or blood pressure response stimuli at any dose or time point. Conclusions: a dose level of 38.5 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 20.8 mg/kg or 1.2 g/day.

4. Respiratory Effects

Mice

In a single dose study, male CD-1 mice (4/dose) were given FTC orally at dose levels of 0, 100, 250, 750 or 1000 mg/kg, then observed for 7 days to collect data on respiratory rate. FTC did not affect respiratory rate at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 81.1 mg/kg or 4.8 g/day.

Rats:

In a single dose study, male CD (SD) rats (4/dose) were given FTC orally at dose levels of 0, 100, 250, 750 or 1000 mg/kg, then observed for 7 days to collect data on respiratory rate. FTC did not affect respiratory rate at any dose. Conclusions: a single dose of 1000 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 162.33 mg/kg or 9.7 g/day.

Dogs:

In a single dose cardiovascular and respiratory effects study, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10 and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more min. Respiratory rate and respiratory minute volume were measured at intervals. FTC did not affect respiratory function

parameters at any dose or time point. Conclusions: a dose level of 38.5 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 20.8 mg/kg or 1.2 g/day.

5. Renal effects

In a single dose renal function study, male Long-Evans derived rats (6/dose) were given FTC orally at dose levels of 0, 30 or 100 mg/kg and urine was collected for 6 hr postdose to measure pH, volume and electrolyte concentrations. FTC did not affect urine output, pH or electrolyte excretion at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 16.2 mg/kg or 974 mg/day.

6. Gastrointestinal Effects

In a single dose gastrointestinal motility study, male ICR mice (10/dose) were given FTC orally at dose levels of 0, 10, 30 or 100 mg/kg, then given a charcoal suspension orally at 1 hr postdose and euthanatized 15 min later to record intestinal transit of charcoal. FTC did not affect gastrointestinal motility at any dose. Conclusions: a single dose of 100 mg/kg may be considered the NOEL. Based on a body surface area factor, an equivalent dose in humans would be 8.1 mg/kg or 486 mg/day.

7. Coagulation System Effects

In an in vitro receptor binding assay, the ability of FTC at 2.47 µg/ml to inhibit binding of radio labeled platelet activating factor (PAF) to PAF receptors was evaluated using platelets for male New Zealand White rabbits. FTC had essentially no effect on binding to the PAF receptor.

VI. PHARMACOKINETICS/TOXICOKINETICS:

Pharmacokinetics/Toxicokinetics Studies Summary:

1. Pharmacokinetics of FTC in Male Mice Following Single Oral and Intravenous Administration, Triangle Pharmaceuticals, Durham, NC, July 25, 1997, (TR1997/0011/00)

2. Pharmacokinetics of 524W91 in Male Mice Following Oral and Intravenous Administration, August 17, 1993, ('93/0003)

3. Pharmacokinetics of 100 mg/kg Oral and IV 524W91 in Male Mice, , August 17, 1993, '93/0004)

4. Pharmacokinetics of FTC in Male Cynomolgus Monkeys Following Single Oral and Intravenous Administration of FTC, Triangle Pharmaceuticals, Durham, NC, July 29, 1997, (TR1997/0020/00)

5. Pharmacokinetics of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration,

   August 17, 1993, (—/309-223/ — /93/0019)

6. Metabolic Disposition and Balance Studies in Male Mice Following Oral Administration of 120 mg/kg [6-^{3}H] 524W91,

   September 2, 1993, (/93/0015)

7. FTC: [^{14}C] TP-0006: A tissue distribution and excretion study in rats, March 2, 2000, (TOX-092/8233)

8. Metabolism and excretion of [^{14}C]-FTC following oral administration to male cynomolgus monkeys (TOX-063/6758-118)

9. Metabolic Disposition of 80 mg/kg Orally Administered [6-^{3}H] 524W91 in Cynomolgus Monkeys, October 1, 1993, (/93/0016)

10. Pharmacokinetics, excretion and tissue distribution of

[^{14}C]-FTC following oral administration to male cynomolgus monkeys (TOX-063/6758-118)

11. toxicokinetics report for a six month oral (gavage) toxicity study in mice given FTC with 3-month interim kill, May 10, 2000 (TOX-022)

12. Protein Binding of FTC in Human, Monkey, Mouse and Rabbit Plasma, ——— July 9, 1993, (—— /93/0025)

Review of Pharmacokinetics/Toxicokinetics Studies:

1. Pharmacokinetics of FTC in Male Mice Following Single Oral and Intravenous Administration, Triangle Pharmaceuticals, Durham, NC, July 25, 1997, (TR1997/0011/00)

Two groups of male CD-1 mice were given a single dose of 600 mg/kg FTC by either oral gavage or iv routes. Blood samples were collected at various times up to 24 hr post-dose (3 mice per time point). Plasma was analyzed for FTC by —— After the iv administration, total body clearance was 1.3 L/kg/hr. Plasma clearance was described by a tri-exponential equation, with a $t_{½}$ of 4.14 hr. The volume of distribution at steady state was 1.1 L/kg. After the oral administration, absorption was rapid, with a T_{max} at 40 min and C_{max} at 139 µg/ml. The absolute bioavailability was 63%.

Comments: Apparent volume of distribution (1.1 L/kg) was numerically greater than total body water volume and suggested that FTC was widely distributed to tissues.

2. Pharmacokinetics of 524W91 in Male Mice Following Oral and Intravenous Administration, —— August 17, 1993, ('93/0003)

Two groups of male CD-1 mice were administered a single 10 mg/kg dose of a solution of 524W91 by either oral gavage or iv routes. Blood samples were collected at scheduled times up to five hr post-does (five mice per time points). Plasma 524W91 was analyzed by a —— method. After the iv administration, total body clearance was 2.33 L/kg/hr. Plasma clearance was bi-exponential, with $t_{½}\alpha$ = 4 min and $t_{½}ß$ = 23 min. The volume of distribution at steady state was 0.89 L/kg. After the oral administration, absorption was rapid, with T_{max} at 25.4 min and the C_{max} at 9.8 µM. The absolute oral bioavailability was 96%.

Comments: Elimination of 524W91 by the mouse is rapid. The total clearance of 2.33 L/kg/hr is close to the estimated renal plasma flow of 2.25 L/kg/hr in a 30 g mouse. The metabolic studies have

shown that 524W91 is primarily eliminated in the urine as unchanged drug. The total clearance is therefore almost entirely due to renal instead of metabolic processes, suggesting that an active secretory system in the renal tubule is able to clear nearly all of 524W91 from the plasma as blood flows through the kidney. 524W91 appears to permeate mouse tissues well, as suggested by the VDss of 0.89 L/kg.

3. Pharmacokinetics of 100 mg/kg Oral and IV 524W91 in Male Mice, August 17, 1993, ('93/0004)

Two groups of male CD-1 mice were given a single dose of 100 mg/kg 524W91 by either oral gavage or iv routes. Blood samples were collected at various times up to 24 hr post-dose (5 mice per time point). Plasma was analyzed for 524W91 by —— After the iv administration, total body clearance was 2.23 L/kg/hr. Plasma clearance was described by a tri-exponential equation, with a $t_{½}\alpha$ of 1.7 min, a $t_{½}ß$ of 15.5 min and a $t_{½}\gamma$ of 82 min. The volume of distribution at steady state was 0.94 L/kg. After the oral administration, absorption was rapid, with a T_{max} at 24.5 min and C_{max} at 89 µM. The absolute bioavailability was 79%.

Comments: The pharmacokinetics and oral bioavailability of 524W91 did not appear to be dose-dependent over the range of 10-100 mg/kg range in mouse.

4. Pharmacokinetics of FTC in Male Cynomolgus Monkeys Following Single Oral and Intravenous Administration of FTC, Triangle Pharmaceuticals, Durham, NC, July 29, 1997, (TR1997/0020/00)

Four male cynomolgus monkeys were administered a single 80 mg/kg dose of FTC by intravenous infusion and nasogastric gavage in a crossover design study. Blood samples were collected at various times up to 24 hr post-dose. Drug plasma concentrations were determined by an —— method. Mean pharmacokinetic parameters of FTC after the iv and oral administrations are shown in Table 1.

Table 1
Mean Pharmacokinetic Parameters of Single Doses of FTC in Male Monkeys Following Intravenous and Oral Administrations

Parameters	80 mg/kg	
	IV	Oral
C_{max} (µg/ml)	-	39.4 ± 4.8

AUC_{∞} (µg*hr/ml)	86.1 ± 17.3	83.6 ± 11.2
CL_T (L/kg/hr)	0.97 ± 0.15	-
Vd_{ss} (L/kg)	0.769 ± 0.07	-
$T_{1/2}\beta$ (hr)	0.775 ± 0.05	0.936 ± 0.09
T_{max} (hr)	-	-
F (%)	-	97.4 ± 6.9

5. Pharmacokinetics of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration, August 17, 1993, (— '309-223 — /93/0019)

Eight male cynomolgus monkeys were administered a single 10 mg/kg or 80 mg/kg dose of 524W91 (4 monkeys each dose) by intravenous infusion and nasogastric gavage in a crossover design study. Drug plasma concentrations were determined by an — method. Mean pharmacokinetic parameters of 524W91 after the iv and oral administrations are shown in Table 2.

Comments: Dose-independent kinetics were observed over the concentration range used in this study.

Table 2
Mean Pharmacokinetic Parameters of Single Doses of 524W91 in Male Monkeys Following Intravenous and Oral Administrations

Parameters	Intravenous		Oral	
	10 mg/kg	80 mg/kg	10 mg/kg	80 mg/kg
C_{max} (µM)	129±25	1093±59	14.0±2	111±34
AUC (µM*hr)	60±11	493±65	37.4±6.5	285±82.6
CL_T (L/kg/hr)	0.7±0.14	0.7±0.08	-	-

Vd_{ss} (L/kg)	0.8±0.02	0.8±0.09	-	-
$T_{½\alpha}$ (hr)	0.06±0.01	0.06±0.01	-	-
$T_{½\beta}$ (hr)	1.00±0.19	1.02±0.13	-	-
T_{max} (hr)	-	-	1.3±0.5	2.3±0.3
F (%)	-	-	62.7±4.3	57.5±11.7
CL/F	-	-	1.1±0.2	1.2±0.31

6. Metabolic Disposition and Balance Studies in Male Mice Following Oral Administration of [6-^{3}H]-524W91 (120 mg/kg), ——— September 2, 1993, /93/0015)

Male CD-1 mice (30-35 g) were dosed orally with 10 ml/kg of 12 mg/ml 524W91 containing 32 µCi/ml of [6-^{3}H]-524W91. Urine samples were collected over 0-24, 24-48 and 48-72 hr periods. Feces were collected over the same intervals. Urine and feces were assayed by liquid scintillation counting ——— . In the feces, 18±3% of the dose was recovered, all as unchanged 524W91. In the urine, 64±7% of the radioactivity was recovered as unchanged 524W91, mostly in the 0-24 hr sample. The average cumulative recovery of radioactivity in the urine over the 72-hr period was 67±7% of the dose. Total recovery of radioactivity excreted in urine and feces was 85±4% of the dose. Three metabolites of 524W91 were measurable in the urine. These metabolites were tentatively identified as: 3742W92 (1.7%) and 3743W92 (2.0%), two isomeric, 3'-sulfoxides of 524W91 and 5-flurocytosine (1.4%). Traces of five other metabolites and a peak tentatively identified as tritiated water were also observed (<1%).

Comments: Nearly all the radioactivity excreted in the urine was excreted within the first 24 hr post-dose, which is consistent with the rapid elimination of 524W91 by the mouse. The 18% of the dose that was recovered as 524W91 in the feces is probably unabsorbed 524W91, since the absolute oral bioavailability of 524W91 in the mouse has been shown to be 79% at 100 mg/kg. Metabolism of 524W91 appeared to be a minor pathway of clearance in the mouse.

7. FTC:[^{14}C]TP-0006: A tissue distribution and excretion study in rats, March 2, 2000, (TOX-092/8233)

Twenty male Sprague Dawley nonpigmented rats (Group 1) and six male Long-Evans pigmented rats (Group 2) received a single 200 mg/kg oral dose containing approximately 135 µCi/kg of FTC:[^{14}C]TP-0006 via gavage. Blood samples were collected from 3

nonpigmented and 1 pigmented rat per time point at 1, 4, 8, 24, 72 and 144 hr post dose, and plasma was harvested. Urine and feces were collected from selected nonpigmented rats at intervals up to 144 hr post dose. Tissue distribution of the labeled FTC was studied using whole-body autoradiography with 3 nonpigmented and 1 pigmented rats at 1, 4, 8, 24, 72 and 144 hr post dose. Results: following a single oral dose of FTC, a summary of the PK parameters in blood and plasma is shown in Table 3. The distribution of radioactivity was rapid, with maximal levels occurring at 1 hr post dose in all non-GI tissues except inguinal lymph nodes (Cmax = 4 hr). Excluding the GI tract and urine, the highest concentrations of the radioactivity were found (in decreasing order) in the renal medulla, renal cortex, liver and urinary bladder. After reaching a Cmax, the concentration of radioactivity declined rapidly and by 24 hr post dose were non-detectable in all tissues except those of the GI tract. Distribution of radioactivity in pigmented rats was similar to nonpigmented rats, indicating that FTC did not bind to melanin to any significant extent. Recovery of total radioactivity in the urine and feces averaged 79.3% and 18.4% of the administered dose, respectively, with 95% of the total radioactivity excreted within 24 hr.

Table 3

Summary of the pharmacokinetic parameters of FTC following a single oral dose (200 mg/kg) of FTC in rats

Groups		Cmax (µg equiv/ml)	Tmax (hr)	T½ (hr
Group 1	Blood	48	1	1.31
	Plasma	51.9	1	2.74
Group 2	Blood	54.4	1	1.18
	Plasma	59.2	1	3.42

8. Metabolism and excretion of [^{14}C]-FTC following oral administration to male cynomolgus monkeys (TOX-063/6758-118)

Four adult male monkeys were given a single oral dose of 200

mg/kg FTC containing approximately 138 µCi of [^{14}C]-FTC (phase 1). Blood, urine and feces were then collected at selected intervals up to 120 hr post dose. After approximately 3 weeks, the four monkeys were dosed as described above (phase 2). Putative metabolites of FTC were identified by ——— and, where possible, ——————— Results: for plasma, urine, feces and selected tissues analyzed, the parent drug was the predominant compound present. In urine, 29.2% of the dose was accounted for as FTC and 11.6% as metabolites, with M1, M2 and M3 together representing 8.4% of the dose. M1 and M2 are putative 3-sulfoxide diastereomers of FTC and M3 is a 2-O-glucuronide of FTC. In feces, 34.4% of the dose was accounted for as FTC and only 1.3% of the dose as metabolites, suggesting incomplete absorption or possible intestinal secretion of FTC in monkeys. Unchanged FTC represented the great majority of radioactivity present in urine (74%) and feces (97%), indicating that FTC is not extensively metabolized.

9. Metabolic Disposition of 80 mg/kg Orally Administered [6-^{3}H]-524W91 in Cynomolgus Monkeys,
——— October 1, 1993, '93/0016)

The metabolism and elimination in urine and feces of 80 mg/kg orally administered [6-^{3}H]-524W91 containing 0.44 mCi/mmole was studied in four female cynomolgus monkeys. Urine samples were collected over 0-8, 8-24, 24-48 and 48-72 hr periods. Feces were collected over the same intervals. The 0-8 and 8-24 hr fecal samples were pooled prior to homogenization. Urine and feces were assayed by liquid scintillation counting ——— After 72 hr, 41% of the dose was excreted into the urine. Most of the urinary excretion occurred (33±9%) in the first 8 hr post-dose sample. Recovery of radioactivity in the feces averaged 33% of the dose after 72 hr. Recovery of radioactivity in the cage wash averaged 10% of dose over the same time period. The overall recovery of dose was 84%. Radiochemical —— analysis of urine and fecal samples showed that unchanged 524W91 was 64% of the recovered dose in the urine and 98% of the feces. The major urinary metabolites constituted 11% of the dose. They were designated as: M200, M350, M950, M1030, M1500 and M1980. M350 co-eluted with 5-fluorocytosine, M950 and M1030 co-eluted with two isomeric 3'-sulfoxides of 524W91, and M1980 co-eluted with the deaminated metabolite of 524W91. Another potential urinary metabolite, 5-fluorouracil, was less than 0.02% of the dose. In the feces, unchanged 524W91 was the only quantifiable compound present. Traces of M950 and M1030 were detectable.

Comments: Metabolism is a minor contributor to clearance of 524W91 in the monkey, since 64% of the radioactivity in the urine was present as parent compound. Approximately 11% of the dose was metabolized to a compound that co-eluted with a 3'-sulfoxide of

524W91.

10. Pharmacokinetics, excretion and tissue distribution of [^{14}C]-FTC following oral administration to male cynomolgus monkeys (TOX-063/6758-118)

Four adult male monkeys were given a single oral dose of 200 mg/kg FTC containing approximately 138 µCi of [^{14}C]-FTC (phase 1). Blood, urine and feces were then collected at selected intervals up to 120 hr post dose. After approximately 3 weeks, the four monkeys were dosed as described above (phase 2). A blood sample was collected at 1 hr post dose, the monkeys were anesthetized, and cerebrospinal fluid was collected. Then the animals were exsanguinated and selected tissues were collected. All samples were analyzed for total radioactivity by liquid scintillation counting. Results: after phase 1 administration, blood and plasma concentrations declined rapidly over time; mean pharmacokinetic parameters are shown in Table 4. The mean total recovery of radioactivity dose in urine, feces, cage wash and cage wipes over the 120 hr collection interval was 84.5%. Urine and feces accounted for a mean of 40.8% and 35.3% of the total radioactive dose, respectively, with 66.4% of the radioactivity excreted within 48 hr post dose. The case wash and cage wipes accounted for an additional 8.31% of the total radioactive dose.

At 1 hr following phase 2 dose administration, with the exception of the gastrointestinal tract tissues (small intestine, large intestine and stomach), the highest mean concentrations of radioactivity in the tissues were observed in the kidneys (596 µg/g) and liver (121 µg/g). The ratios for radioactivity concentrations found in the brain, cerebrospinal fluid, bone, aqueous humor and eyes as compared to blood were 0.028, 0.031, 0.039, 0.063 and 0.098, respectively indicating distribution of radioactivity into these tissues ranging from 2.17 to 7.53 µg/g.

Table 4

Mean plasma pharmacokinetic parameters of a single oral dose of FTC in male monkeys following oral administration

Parameters	200 mg/kg, oral
C_{max} (µg equiv/g)	63.5
$AUC_{0-\infty}$ (µg equiv*hr/g)	272
$T_{1/2\beta}$ (hr)	8.05
T_{max} (hr)	1
elimination rate constant (1/hr)	0.09

11. toxicokinetics report for a six month oral (gavage) toxicity study in mice given FTC with 3-month interim kill, May 10, 2000 (TOX022)

Groups of male and female CD-1 mice (10 animals/sex/group; strain: Crl: CD-1(ICR)BR) were administered FTC via oral gavage at dose levels of 0 (0.5% methylcellulose, vehicle control) 167 (low), 500 (mid) or 1500 mg/kg/day (high). At 3 and 6 months of exposure, plasma samples were collected at predose, 0.5, 1, 2, 5 and 8 hr after the daily dose. The plasma samples were analyzed for concentrations of FTC using a validated — method.
Results: are summarized in Table 5. FTC was rapidly absorbed with Cmax occurring by 30-60 min post dosing. The systemic exposure to FTC and the peak plasma concentrations increased linearly as the dose increased from 167 to 1500 mg/kg/day. FTC was eliminated from the plasma with T½ of 3-4 hr. Cmax and AUC estimates in both sex groups obtained at 6-month were higher than those obtained at 3-month, especially for the high dose level.

Table 5
Mean pharmacokinetic parameters of FTC for a 6-month oral gavage toxicity study in mice

Dose (mg/kg/day)		3-month		6-month	
		Cmax (µg/ml)	$AUC_{0-\infty}$ (hr*µg/ml)	Cmax (µg/ml)	$AUC_{0-\infty}$ (hr*µg/ml)
167	male	44	63.8	41.7	82.3
	female	37.2	67.9	48.6	93.1
500	male	128	181.4	87.3	248.4
	female	88.8	209.6	111.7	284.4
1500	male	225	513.4	310.9	732.1
	female	187.1	569.5	306	899.4

12. Protein Binding of 524W91 in Human, Monkey, Mouse and Rabbit Plasma, —, July 9, 1993, — /93/0025)

The binding of [^{3}H]-524W91 to human, monkey, mouse, and rabbit plasma proteins was determined over a concentration range of 0.02-200 µg/ml by equilibrium dialysis at 37°C. The mean percentage bound for all species studied was less than or equal

to 3.6%, with no indication of concentration dependency.

Comments: Because of the low extent of binding to human plasma at therapeutic concentrations, drug interactions mediated by protein binding displacement are not expected with 524W91.

V. GENERAL TOXICOLOGY:

General Toxicology Studies Summary: The following studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practices Regulations.

Single dose studies:

1. FTC: An acute iv and oral toxicity study in mice and acute oral toxicity study in rats, Lot # TP-006/96/001, May 1999, '05/1999)

2. An Acute Oral Toxicity Study in Mice, Batch # 92/1271-014-A, Burroughs June 1, 1993, '93/0020)*

3. An Acute Intravenous Toxicity Study in Mice, Batch # 92/1271-014-A, June 17, 1993, '93/0023)

4. An Acute Oral Toxicity Study in Rats, Batch # 92/1271-014-A, June 1, 1993, /93/0021)

5. Acute Intravenous Toxicity Study in Rats, Batch # 92/1271-014-A, June 18, 1993, '93/0024)*

Multiple dose studies:

6. A 14-Day Oral Toxicity Study in Mice, Batch # BP-499-55-1, Triangle Pharmaceuticals, Durham, NC, July 23, 1997, (IUW00701)*

7. 14-Day Oral Bridging Toxicity Study in Mice, Batch # TP-0006 Triangle Pharmaceuticals, Durham, NC, September 21, 2000, (TOX113/Study No. 814-004)

8. A Thirty Day Oral Toxicity Study in Mice, Batch # 92/1271-014-A, August 8, 1993, (/93/0029)*

9. A one-month mouse oral toxicity study of FTC containing FTU and two potential process impurities, Lot # TP-0006-00137, March 21, 2001, (TOX-118/670A-001-032-00)*

10. Six-month oral toxicity study (with a three month interim sacrifice) in mice given 524W91, Reference # 93/5275-006-S, June 3, 1994, /94/0030/TOX628)

11. Six-month oral toxicity study (with a three month interim sacrifice) in mice given 524W91, Reference # 93/5275-006-S, June 3, 1994, (/94/0030/TOX628)*

12. Six month oral toxicity study in mice given FTC with a 3-month interim kill: 3-month interim report, Batch # 27127, October 2, 1998, (TOX-022/IUW01001)*

13. A 3-month oral toxicity study for bioassay dose selection in CD rats, Lot No. TP-0006-00094, November 3, 2000 (TOX-097/814-003)*

14. A Thirty Day Oral Toxicity Study in Cynomolgus Monkeys, Batch # 92/1271-014-A, November 8, 1993, (/93/0030/ 309-227)*

15. A 3-Month Oral Toxicity Study in Cynomolgus Monkeys Given 524W91, Lot # 912045, June 23, 1994 TOX 627 /94/0036/ 309-229)*

16. FTC: 52-Week oral toxicity study in cynomolgus monkeys with