a 4-week recovery period, Batch # TP-0006/96/CC, Triangle Pharmaceuticals, Durham, NC, November 8, 1999, (TOX-032/Document code: 3262)*

Review of general toxicology studies:

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)

Groups of male and female mice (weights: 27-29 g; age: 7-8 weeks) and rats (weights: 251-258 g) were administered FTC according to a design shown in Table 1. After dosing, behavior changes of the animals were observed daily for 14 days.

Table 1
Experimental design of the mice and rats acute toxicity study

Study group	Dose (mg/kg)	Frequency (daily	Total dose (mg/kg)	Dose volume (ml/kg)	No. of animals	
					male	female
Mouse iv	350	2	700	10	10	10
Mouse oral	525	2	1050	15	10	10
Rat oral	700	2	1400	20	10	10

Results: no animal died during the study. There were no signs of toxicity. There were no changes in body weights or feed consumption.

Comments: 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.

2. An Acute Oral Toxicity Study in Mice, Batch # 92/1271-014-A, June 1, 1993,

'93/0020)*

A group of 5 male and 5 female (weight: 18-22 g; age: 30-33 days) CD-1 mice were fed via oral gavage a single 4 g/kg dose of 524W91 in 0.5% methylcellulose. There was no vehicle control group in this study. The animals were observed for signs of ill health throughout the study and individual body weights were recorded at days 1, 7, 13 and 14. All mice were killed on postdose day 14 and necropsies were performed. Results: there were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. Conclusion: the median lethal oral dose was greater than 4 g/kg.

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

A group of 5 male and 5 female (weight: 19-24 g; age: 30 days) 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 was no vehicle control group in this study. The animals were observed for signs of ill health throughout the study and individual body weights were recorded at days 1, 7, 13 and 14. All mice were killed on postdose day 14 and necropsies were performed. Results: there were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. Conclusion: the median lethal iv dose was greater than 200 mg/kg.

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

A group of 5 male and 5 female (weight: 106-122 g; age: 41 days) CD rats were fed via oral gavage a single 4 g/kg dose of 524W91 in 0.5% methylcellulose. There was no vehicle control group in this study. The animals were observed for signs of ill health throughout the study and individual body weights were recorded at days 1, 7, 13 and 14. All rats were killed on postdose day 14 and necropsies were performed. Results: there were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. Conclusion: the median lethal oral dose was greater than 4 g/kg.

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

A group of 5 male and 5 female (weight: 126-148 g; age: 39 days) 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. The animals were observed

for signs of ill health throughout the study and individual body weights were recorded at days 1, 7, 13 and 14. All rats were killed on postdose day 14 and necropsies were performed. Results: there were no deaths; no clinical signs were noted throughout the study; and no treatment-related gross findings were noted. Conclusion: the median lethal iv dose was greater than 200 mg/kg.

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)*

Groups of male and female CD-1 mice (weight: 19-25 g; age: 3-4 weeks; 15 animals/sex/group; strain: VAF/plus) 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. The dose was given as divided dose with 6 hr apart between the morning and afternoon doses. Five male and female mice per group were assigned to a two-week recovery period. For drug plasma level determinations on days 2 and 14, groups (low, mid or high) of male and female mice (8 animal/sex/group) were also maintained. Mortality: there were two deaths during the dosing phase of the study. One male (control) and one male (high) died as result of dosing technique. No drug-related clinical signs were noted. No drug-related change on urinalysis parameters, ocular examination, body weight, food consumption, blood chemistry, organ weight, clinical pathology or histopathology were noted at the high dose. Drug Plasma Concentrations: at the high dose, mean concentrations were 318 and 358 µg/ml for males and females, respectively, on day 2; on day 14, they were 389 and 405 µg/ml.

Comments: 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.

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

Two groups of male and female CD-1 mice (weight: 21 to 28 g; age: 6 weeks; 8 animals/sex/group; strain: Crl: CD-1(ICR)BR) 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. Results: No adverse clinical findings were seen. No effect of treatment on clinical findings was observed. No effect of treatment on body weight or food consumption was observed. No effect of treatment

on hematology values was seen. Clinical Chemistry: Males and females at the 120 mg/kg/day dose level had slightly lower average total protein (10%), albumin (8%) and globulin (12%) and marginally higher urea nitrogen (10%) values when compared to controls. These differences did not reach statistical significance. There were no drug-related macroscopic, organ weights or microscopic changes observed in these mice.

Comments: 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.

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

Groups of male and female mice (weight: 19-24 g; age: 48-52 days; 10-14 animals/sex/group) 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. Four male and 4 female mice per group were randomly assigned to a two-week recovery period. For drug plasma level determinations on days 3 and 32, groups (low, mid or high) of male and female mice (38 animal/sex/group) were also maintained. Mortality: one low dose female died due to misdosing and 4 males and 2 females (high) died as result of the treatment. No drug-related clinical signs were noted. No drug-related change on urinalysis parameters, ocular examination, body weight and food consumption were noted. Hematology: at 32 days postdose, hematocrit (HCT) and hemoglobin (Hb) were reduced in females (high). Total RBC count was decreased in both sexes (high). These changes were accompanied by an increase in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red blood cell distribution width (RDW) in both males and females. At the end of two-week recovery period, the changes noted in high dose females had reversed. In high dose males, there was a further decline in HCT and RBC, while some improvement was noted in MCV and RDW. Organ weights: significant increases in pituitary, heart, spleen and ovary (high, females) and spleen (high, males), and decreases in testes and thymus (high, males) were noted. No treatment-related gross pathology findings were noted. There were no accompanying histopathologic correlates noted. No ultrastructural changes were seen in heart or skeletal muscle. Drug Plasma Concentrations: the C_{max} and AUC increased with the dose and there was no evidence of accumulation. The group mean data are presented in Table 2.

Table 2

Plasma toxicokinetic parameters of 524W91 in the 30-day mice study

Dose (mg/kg/day)	Day 3				Day 32			
	C_{max} (µM)		AUC (µM*hr)		C_{max} (µM)		AUC (µM*hr)	
	Male	Female	Male	Female	Male	Female	Male	Female
120	165	211	316	336	208	157	416	299
600	810	791	1660	1320	887	853	2020	1230
3000	3030	3000	7280	6920	2520	2730	7830	6130

Comments: 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. The daily administration of 3000 mg/kg/day resulted in decreased RBC, Hb and HCT and increased MCV, MCH and RDW.

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)*

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. Results: no drug related mortality occurred and there were no clinical signs noted during the treatment. Hematology: statistically significant increased hemoglobin and hematocrit values were recorded for males (high) and increased MCV was recorded for female mice (high). Organ weights: statistically significant heavier kidneys (both absolute and relative) were recorded for male mice (high). Histopathology: there were no findings associated with increased male kidney weights.

Comments: 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.

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)

Groups of male and female ——— : CD-1 mice (age: 62-67

days; 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. Hematology: a slight reduction in total erythrocyte count was seen in both sexes (mid and high). Slight increases in mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width were noted in males and females (high). Urinalysis: a dose-related increase in urine quantity was noted in male and female mice on dose days 86-87. The change was unaccompanied by any alterations in specific gravity, pH or urinary electrolytes. No treatment-related microscopic changes were seen in the kidney to correlate with the urinalysis findings. Drug Plasma Concentrations: plasma concentration of 524W91 increased with the dose (Table 3).

Table 3
524W91 Plasma Concentrations in Mice After 3-Month of Oral Dosing with 524W91

Dose (mg/kg/day)	Cmax (µM)		AUC (µM*hr)	
	Male	Female	Male	Female
120	139.7	146.9	439.9	363.9
600	781.9	643.7	2189.7	1630.3
3000	1270	1913	7024.1	5796.7

Comments: The daily administration of 3000 mg/kg/day of 524W91 resulted in a decrease in RBC and an increase in MCV, MCH and RDW. In the 3-month interim sacrifice study, 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.

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)*

Groups of male and female ——— CD-1 mice (age: 62-67 days; 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 six months. Before the start of dosing, five animals of each sex were randomly selected from each dose group for a post-dose recovery period of approximately 3 weeks. These mice were bled for clinical pathology at the end of the recovery period. The following organs

were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, salivary gland, spleen, testes/epididymides, thymus, uterus with fallopian tubes, and pituitary. Histopathological examination was performed on the following organs/tissues: adrenal glands, aorta, bone marrow, brain, cervix, eyes, femoral/tibial joint, gall bladder, harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs (including main stem bronchi), mesenteric lymph nodes, oesophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, site of mammary gland, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, submandibular lymph node, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions. Results: five male (1 control, 1 low, 1 mid and 2 high) and 1 female mice (high) died during the study. The cause of death could not be determined for two mice (1 each in low and mid); the others died due to gavage accidents. There were no treatment-related clinical signs. Hematology: very slight and reversible changes in total erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) relative to control values were observed in male and female mice (high). These changes were present after approximately 3 months of the dosing and were no greater in severity after 6 months of the treatment. Urinalysis: a dose-related increase in urine quantity was noted in male and female mice on dose day 179. The change was unaccompanied by any alterations in specific gravity, pH or urinary electrolytes. No treatment-related microscopic changes were seen in the kidney to correlate with the urinalysis findings. Drug Plasma Concentrations: plasma concentration of 524W91 increased with the dose (Table 4). Females had statistically significantly lower plasma AUC values of the test compound than did males at all dose levels. 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.

Table 4
524W91 Plasma Concentrations in Mice After 6-Months of Oral Dosing with 524W91

Dose (mg/kg/day)	Cmax (μM)		AUC_{0-24} (μM*hr)	
	Male	Female	Male	Female
120	279	181	686	467

600	613	760	2369	1985
3000	2567	2034	8195	6266

Comments: 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.

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

Four groups of male and female mice (strain: Crl: CD-1 (ICR)BR; age: 4-5 weeks; 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. At the end of this period, a maximum of 10 animals/sex/group were killed and the remaining animals continued to receive the dosing for a further 13 weeks. At the end of 26 weeks, 5 animals/sex/group commenced a one-month recovery period to investigate the reversibility of any findings. A further 40 animals/sex/group (toxicokinetics arm) received the above mentioned treatment for 13 weeks whereupon 3 animals/sex/group were bled at 6 different time points for toxicokinetic samples and were subsequently killed in weeks 13 and 26. The plasma samples were analyzed for FTC by an validated [redacted] method. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, salivary gland, spleen, testes/epididymides, thymus, uterus with fallopian tubes, and pituitary. Histopathological examination was performed on the following organs/tissues: adrenal glands, aorta, bone marrow, brain, cervix, eyes, femoral/tibial joint, gall bladder, harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs (including main stem bronchi), mesenteric lymph nodes, oesophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, site of mammary gland, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, submandibular lymph node, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions Results: there were no clinical observations considered to be due to FTC administration. Mortality: there were three deaths during the first three months of the study. One control male was killed on day 7. This animal did not show any notable findings at necropsy or under histopathological evaluation (protocol organs

and tissues). Two satellite animals were found dead, one male on day 25 and one male on day 85. There were no drug-related effects on the following parameters: Body weights, food consumption, ophthalmoscopy, hematology, blood chemistry, necropsy findings, organ weights or histopathological examination. Toxicokinetics: mean toxicokinetic parameters 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.

6-month report: there were no drug-related effects on the following parameters: Body weights, food consumption, ophthalmoscopy, blood chemistry, necropsy findings, organ weights or histopathological examination. Hematology: a small statistically significant ($p<0.05$) increase was seen for MCH and MCV in the high dose animals.

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_{0-24} (hr*µg/ml)	Cmax (µg/ml)	AUC_{0-24} (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

Comments: 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.

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

Four groups of male and female CD rats (strain: Crl:CD(SD)IGS BR; age: 5 weeks; weight: 159-199 g males and 120 to 155 females; 10 animals/sex/group + 18 animals/sex/toxicokinetics 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. Blood samples were obtained at predose (0.5 hr), 1, 2, 4, 8 and 12 hr postdose on days 2 and 90. The plasma samples were analyzed for FTC by a validated [redacted] method. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, salivary gland, spleen, testes/epididymides, thymus, uterus with fallopian tubes, and pituitary. Histopathological examination was performed on the following organs/tissues: adrenal glands, aorta, bone marrow, brain, cervix, eyes, femoral/tibial joint, gall bladder, harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs (including main stem bronchi), mesenteric lymph nodes, oesophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, site of mammary gland, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, submandibular lymph node, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions Results: all animals survived to their scheduled termination after 3 months of study. No drug related clinical findings were observed during the study. Body weights: are summarized in Table 6. There was no statistical significance in the body weights. No effect of treatment on body weight was seen.

Table 6

Mean body weight values at week 13 in the 3-month oral gavage toxicology study in CD rats

Dose (mg/kg/day) Group		Mean body weight (g)	
		Mean	% change from control
0 (control)	male	536	-
	female	280	-
120 (low)	male	537	+0.2
	female	285	+1.8
600 (mid)	male	544	+1.5
	female	273	-2.5

3000 (high)	male	558	+4.1
	female	278	-0.7

Food consumption: values are summarized in Table 7. The average food consumption for the treated groups was similar to the controls. Any slight differences noted were not considered to be biologically meaningful. No effect of treatment on food consumption was observed.

Table 7
Average food consumption values for weeks 1 through 13 in the 3-month oral gavage toxicology study in CD rats

Dose (mg/kg/day) Group		Average food consumption (g/animal/day)	
		Mean	% change from control
0 (control)	male	30	-
	female	19.7	-
120 (low)	male	28.7	-4.3
	female	19.5	-1
600 (mid)	male	28.8	-4
	female	19.5	-1
3000 (high)	male	30.2	-0.7
	female	20.4	+3.6

Hematology, blood chemistry, necropsy findings, organ weights or histopathological examination: a very slight but statistically significant decreases in erythrocyte and hemoglobin, and blood urea nitrogen in females (high) was observed. There were no drug related macroscopic or microscopic changes noted in either sex at any dose level. There were statistically significant decreases in pituitary gland and thyroid/parathyroid gland weights in males

(high), and male and female (high) animals. Drug Plasma Concentrations: a summary of FTC toxicokinetic parameters is presented in Table 8. FTC was rapidly absorbed and values for Tmax (1-2 hr) tended to increase with increasing dose, suggesting a slower oral absorption rate at higher doses. Female rats tended to absorb FTC more rapidly than males, with observed Cmax values on day 90 being approximately 20-50% higher in females. Observed plasma concentrations and systemic exposure of FTC were higher on day 90 compared to those on day 2. Dose normalized (AUC) values remained relatively constant with increasing dose, suggesting a linear relationship between exposure and daily dose of FTC in this study.

Table 8
Mean toxicokinetic parameters from the 13-week rat toxicology study.

Dose (mg/kg/day) & Sex		C_{max} (µg/ml)		Tmax (hr)		T½ (hr)		AUC_{0-24} (µg*hr/ml)	
		day 2	day 90	day 2	day 90	day 2	day 90	day 2	day 90
120	♀	18.0	21	1	1	2.36	6.5	55.5	69
	♂	15.3	17.5	1	1	2.41	5.2	61.63	66
600	♀	73.1	120	1	1	2.38	3.5	260.37	362
	♂	59.2	78	1	1	3.58	3.8	295.96	329
3000	♀	225	323	2	2	2.67	4.5	1357	1646
	♂	211	222	2	2	3.8	4.5	1413	1276

Comments: No effects were seen at the low or mid dose. A daily dose of 600 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 97.4 mg/kg/day or 5.8 g/day for a 60 kg person.

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

Groups of male and female cynomolgus monkeys (weight: 2.1-3.9 kg;

age: 2.5-7 yr; 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. The total dose was administered in two equal portions approximately 6 hr apart. In addition, there was a recovery period of approximately 3 weeks for selected animals (2 animals/sex/group). Blood samples (2 ml) were collected from all animals on days 3 and 27 at 0, 0.5, 1, 2, 3, 5 and 6 hr following the first dose in the morning. Cerebrospinal fluid (CSF) samples were obtained from each animal during the last dosing week at 1 hr following the morning dose. Results: there were no deaths or illness noted during the course of the study. Drug-related clinical signs included a higher incidence of soft feces in the high dose males. At the high dose, there were no changes in body weights, appetite, clinical pathology values, gross necropsy findings, ophthalmology, organ weight data or electrocardiography, histopathology, neuropathology findings, which were suggestive of a treatment-related effect. Drug Plasma Concentrations: the C_{max} and AUC increased with the dose and there was no evidence of accumulation. The group mean data are presented in Table 9. Mean drug CSF concentrations at low, mid and high dose levels were 4.3%, 3.7% and 3.7% of plasma levels, respectively.

Table 9
Toxicokinetic parameters of 524W91 in the 30-day toxicology study in monkeys

Dose (mg/kg/day)	Day 3				Day 27			
	C_{max} (μM)		AUC (μM*hr)		C_{max} (μM)		AUC (μM*hr)	
	Male	Female	Male	Female	Male	Female	Male	Female
80	59	64	150	153	50	52	128	136
400	236	267	687	688	258	255	793	664
2000	786	826	2553	3095	815	774	2954	2959

Comments: 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. The daily administration of 2000 mg/kg/day resulted in a higher incidence of soft feces. No effects were seen at the low or mid dose.

15. A 3-Month Oral Toxicity Study in Cynomolgus Monkeys Given 524W91, Lot # 912045,

June 23, 1994

— TOX 627, — /94/0036, ——-309-229)*

Groups of male and female cynomolgus monkeys (weight: 1.9-3.3 kg; age: 2-5.2 yr; 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. The total dose was administered in two equal portions approximately 6 hr apart. In addition, there was a recovery period of approximately 3 weeks for selected animals (2 animals/sex/group). Blood samples (2 ml) were collected from all animals on days 3 and 87 at 0, 1, 2, 3, 4 and 6 hr following the first dose in the morning. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, seminal vesicles, spleen, testes/epididymides, thymus, thyroid and uterus. Histopathological examination was performed on the following organs/tissues: adrenal glands, aorta, bone marrow, brain, cervix, eyes, femoral/tibial joint, gall bladder, harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs (including main stem bronchi), mesenteric lymph nodes, oesophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, site of mammary gland, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, submandibular lymph node, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder and uterus. Results: there were no deaths noted during the course of the study. One male (high) was noted to have emesis immediately postdose during weeks 1 and 5. There were no changes in body weights. Hematology: at week 1, significant decreases were observed in the mean values for activated partial thromboplastin time (mid) females and eosinophils (high) females. There were no other effects observed at the low or mid dose. At the high dose, no adverse effects on clinical signs, appetite changes, body weight, ophthalmology, electron cardiography, neurophysiology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and histopathology were seen. Drug Plasma Concentrations: the C_{max} — and AUC increased with the dose and there was no evidence of accumulation. The group mean data are presented in Table 10.

Table 10

Plasma toxicokinetic parameters of 524W91 in the 3- month toxicology study in monkeys

Dose (mg/kg/day)	Day 3				Day 87			
	C_{max} (µM)		AUC (µM*hr)		C_{max} (µM)		AUC (µM*hr)	
	Male	Female	Male	Female	Male	Female	Male	Female

40	21	24.5	49.2	54.2	21.3	24.1	52.8	58.7
200	117	92.8	287	210	105	93.6	262	221
1000	408	374	1339	942	474	382	1630	1109

Comments: A daily dose of 1000 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent dose in humans would be 333 mg/kg/day or 20 g/day for a 60 kg person.

16. FTC: 52-Week oral toxicity study in cynomolgus monkeys with a 4-week recovery period, Batch # TP-0006/96/CC, Triangle Pharmaceuticals, Durham, NC, November 8, 1999, (TOX-032/Document code: 3262)*

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 a 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. Serial blood samples were collected for toxicokinetic determinations on day 1 and during weeks 13, 26 and 52 prior to the morning dose and at 0.5,1, 2, 4 and 6 hr after the first dose. The plasma samples were analyzed for FTC by a validated —— method. After 13 weeks of treatment, 2 animals/sex (controls and high) were necropsied. After 52 weeks of treatment, 4 animals/sex/group were necropsied. Surviving animals (controls and high; 2 animals/sex/group) were necropsied following a 4-week recovery period. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, seminal vesicles, spleen, testes/epididymides, thymus, thyroid and uterus. Histopathological examination was performed on the following organs/tissues: adrenal glands, aorta, bone marrow, brain, cervix, eyes, femoral/tibial joint, gall bladder, harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs (including main stem bronchi), mesenteric lymph nodes, oesophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, site of mammary gland, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, submandibular lymph node, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder and uterus. Results: there were no clinical observations considered to be due to FTC administration. There were no drug-related effects on the following parameters: Body weights, food consumption, ophthalmoscopy, neurophysiologic, electrocardiographic, blood chemistry, necropsy findings, organ weights or histopathological examination. A small (not statistically significant) decrease in mean erythrocyte counts and a significant increase in mean corpuscular hemoglobin values were observed in female monkeys (high). Drug Plasma Concentrations: a summary of FTC pharmacokinetic parameters is

presented in Table 1. FTC was rapidly and well absorbed following the oral administration. Plasma FTC was eliminated with a T½ of 2-4 hr in monkeys. There were no major differences in FTC plasma exposure between male and female monkeys. Systemic exposure to FTC increased linearly with dose over the dose range of 50-500 mg/kg/day in both male and female monkeys.

Table 1

Mean pharmacokinetic parameters of FTC during 52-week of dosing in monkeys

Group; dosage (mg/kg/day)		AUC_{0-24} (µg*hr/ml)				Cmax (µg/ml)			
		drug weeks				drug weeks			
		Day 0	13	26	52	day 0	13	26	52
low 50	♂	-	16.1	17	18.1	3.6	3.7	3.7	4.3
	♀	-	18.1	22.9	33.3	1.5	2.9	4.8	7.3
mid 200	♂	-	75	103	97	12.1	11.1	15.6	17.4
	♀	-	100	120	98	14.2	16.9	24.8	21.3
high 500	♂	-	266	342	274	31.6	42	51	51
	♀	-	196	321	238	37	27	39	38

- : not available

Comments: 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.

VI. GENETIC TOXICOLOGY

Genetic toxicology studies summary: The following studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practices Regulations.

1. Mutagenicity Test on FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay (Ames Test), Batch # TP-0006/96/L, , July 30, 1997, study # 18637-0-409R)*

2. In vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay), Lot # BK-512-96-2, . June 23, 1997, (G97BG07.702)*

3. In vivo Mammalian Erythrocyte Micronucleus Test, Lot # BK-512-96-2, August 14, 1997, (G97BG07.123M)*

4. Mutagenicity Test on 524W91 in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test), Batch # 92/1271-014-A, , April 27, 1993, /93/0031/ 15437-0-401)*

Review of genetic toxicology studies:

1. Mutagenicity Test on FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay (Ames Test), Batch # TP-0006/96/L, July 30, 1997, (study # 18637-0-409R)*

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. The S9 homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1250 at a dose level of 500 mg/kg. The concentrations tested, along with vehicle and positive control substances, were 100, 333, 1,000, 3,300 and 5,000 µg/plate. FTC did not cause a positive increase in the number of revertants per plate in any of the tester stains in either the presence or absence of metabolic activation. Conclusion: in the Ames assay, FTC exerted no detectable mutagenic activity.

2. In vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay), Lot # BK-512-96-2, June 23, 1997, (G97BG07.702)*

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. A preliminary experiment was used to select FTC concentrations ranging from 100 to 5000 µg/ml for testing. Based on results of the preliminary experiments, cells at concentrations of 1000, 2000, 3000, 4000 and 5000 µg/ml were cloned and evaluated with and without S9. Results: no cloned culture exhibited mutant frequency. There was no dose-response curve. No visible precipitate was present at any concentration in the treatment medium. No toxicity was observed in the cloned cultures. Conclusions: under the conditions of this study, FTC was found to be negative in the mouse lymphoma mutagenesis assay.

3. In vivo Mammalian Erythrocyte Micronucleus Test, Lot # BK-512-96-2, August 14, 1997, (G97BG07.123M)*

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 micro nucleated polychromatic erythrocytes in bone marrow. Bone marrow cells were collected 24 and 48 hr after treatment and were examined for micro nucleated polychromatic erythrocytes. Results: no significant increase ($p=0.05$) in micro nucleated polychromatic erythrocytes was observed in the treated groups when compared to the controls. Conclusions: the results of the assay indicated that FTC did not induce a significant increase in micro nucleated polychromatic erythrocytes in male mice. FTC was concluded to be negative in the micronucleus assay.

4. Mutagenicity Test on 524W91 in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test), Batch # 92/1271-014-A, April 27, 1993, '93/0031/ 15437-0-401)*

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. For the Salmonella plate incorporation assay, no gene mutations were detected in any strain, with or without metabolic activation, at concentrations between 100 and 5000 µg per plate. The same negative pattern occurred in the Salmonella preincubation assay at the above concentrations. Conclusion: in

the Ames assay, 524W91 exerted no detectable mutagenic activity.

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VII. CARCINOGENICITY

1. FTC mouse protocol: Two-year oral oncogenicity study in CD-1 mice (TOX-109)

2. FTC rat protocol: Two-year oral oncogenicity study in CD rats (TOX-108)

Review of carcinogenicity study protocols:

1. FTC mouse protocol: Two-year oral oncogenicity study in CD-1

mice (TOX-109)

Groups of male and female CD-1 mice (age: 6 weeks; main: 60 mice/sex/group; satellite: 40 mice/sex group + sentinels 18/sex) will be administered FTC via oral gavage (10 ml/kg) at dose levels of 0 (0.5% methylcellulose, vehicle control), 75 (low), 250 (mid) or 750 mg/kg/day (high) for a period of 104 weeks. The satellite animals will be used for drug absorption studies; blood samples will be collected at predose, 0.5, 1, 4, 12 and 24 hr post dose in weeks 2 and 26. At 6-month intervals (6, 12, 18 and 24), the first 3 surviving sentinel mice/sex will be tested for fecal parasites and serology.

Dose selection and justification: The treatment levels (0, 75, 250 or 750 mg/kg/day) were chosen based on a 6-month oral gavage toxicity study in mice. In the study, groups of male and female mice received FTC at dose levels of 0 (control), 167 (low), 500 (mid) or 1500 (high) mg/kg/day once daily for 26 weeks. All doses were well tolerated. There were no treatment related histopathologic alterations. Toxicokinetics showed AUC_{0-24} values of 73 ♂, 81 ♀ with an average of 77 µg*hr/ml at the low dose; 215 ♂, 247 ♀ with an average of 231 µg*hr/ml at the mid dose; and 623 ♂, 734 ♀ with an average of 678.5 µg*hr/ml at the high dose with little differences between the sexes.

The pharmacokinetics characteristics of FTC were similar in mice and humans: The data provide no evidence that the disposition of FTC in humans differs significantly from that in mice in terms of the metabolic pathways, the extent of metabolism or the extent of protein binding.

In the proposed protocol for the assessment of tumorigenic effects of FTC in the mouse, the sponsor is proposing dose levels of 0, 75, 250 or 750 mg/kg/day. 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 750 mg/kg/day (AUC_{0-24} = 339.25 µg*hr/ml extrapolated from the high dose) is expected to provide systemic exposures > 30 times human exposure in the case of both male (33.6 times) and female (39.6 times) mice. Based on the equivalent body surface area factor, the high dose (750 mg/kg/day) in mice is approximately 18 times higher than the maximum dose being utilized in the clinic. 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.

2. FTC rat protocol: Two-year oral oncogenicity study in CD rats (TOX-108)

Groups of male and female CD rats [age: 6 weeks; strain: Crl:CD(SD)IGS BR; 60 rats/sex/group; satellite: 20 rats/sex group] will be administered FTC via oral gavage (10 ml/kg) at dose levels of 0 (0.5% methylcellulose, vehicle control), 60 (low), 200 (mid) or 600 mg/kg/day (high) for a period of 104 weeks. The satellite animals will be used for drug absorption studies; blood samples will be collected at predose, 0.5, 1, 4, 12 and 24 hr post dose in weeks 2 and 26.

Dose selection and justification: The treatment levels (0, 60, 200 or 600 mg/kg/day) were chosen based on a 90-day oral gavage toxicity study in rats. In the study, groups of male and female rats received FTC at dose levels of 0 (control), 120 (low), 600 (mid) or 3000 (high) mg/kg/day once daily for 13 weeks. All doses were well tolerated. There were no treatment related histopathologic alterations. Toxicokinetics showed AUC_{0-24} values of 66 ♂, 69 ♀ with an average of 67.5 µg*hr/ml at the low dose; 329 ♂, 362 ♀ with an average of 345.5 µg*hr/ml at the mid dose; and 1276 ♂, 1646 ♀ with an average of 1461 µg*hr/m at the high) with little differences between the sexes.

FTC was negative in a battery of genotoxicity assays. The pharmacokinetic characteristics of FTC were similar in rats and humans: The data provide no evidence that the disposition of FTC in humans differs significantly from that in rats in terms of the metabolic pathways, the extent of metabolism or the extent of protein binding.
In the proposed protocol for the assessment of tumorigenic effects of FTC in the rat, the sponsor is proposing dose levels of 0, 60, 200 or 600 mg/kg/day. 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. Based on the equivalent body surface area factor, the high dose (600 mg/kg/day) in rats is approximately 29 times higher than the maximum dose being utilized in the clinic.

VIII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Summary of reproductive and developmental toxicology studies: The following studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practices Regulations.

1. FTC: Study of fertility and early embryonic development in CD-1 mice (Segment I), Lot # TP-0006/96/DD, Triangle Pharmaceuticals, Durham, NC, January 15, 1999, (TOX-036/Document No. 4436)*

2. Study of fertility male rats (Segment I) given 524W91 by gavage, Batch # 92/1271-014-A, August 8, 1993, (— /93/0029)

3. A study of the effects of FTC on embryo/fetal development in mice, Lot # 3821A, December 30, 1998, (TOX-037/ — 345003/Document No. 3566)*

4. Toxicokinetic study to determine fetal exposures in CD-1 mice given FTC orally (gavage), November 3, 2000 (TOX103/ — 345005)

5. **A study of the effects on embryo/fetal development in rabbits, lot # TP-0006/96/DDD, June 16, 2000, (TOX038/ 345004)***

6. **Pre- and postnatal development in CD-1 mice (Segment III), Lot # TP-0006/96/DD, Triangle Pharmaceuticals, Durham, NC, March 11, 1999, (TOX-039/Document No. 5063)***

Review of reproductive and developmental toxicology studies:

1. FTC: Study of fertility and early embryonic development in CD-1 mice (Segment I), Lot # TP-0006/96/DD, Triangle Pharmaceuticals, Durham, NC, January 15, 1999, (TOX-036/Document No. 4436)*

Groups of male and female mice (strain: Crl: CD-1 (ICR)BR; 20-21 animals/sex/group; age: 51 days) 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. Results: there were no treatment-related effects at any dose for body weights, body weight changes, feed consumption or clinical observations in the pre-breed period for either sex or for female mice during gestation. There was no evidence of F_0 male or female reproductive toxicity: no effects on male or female mating or fertility indices or on F_0 male seminal parameters (epididymal sperm number and motility). There were no treatment-related findings at gross necropsy for either F_0 males or females. Embryonic development was unaffected by FTC.

Comments: No effects were seen at the low, mid or high dose. 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.

2. FTC: Study of fertility male rats (Segment I) given 524W91 by gavage, Batch # 92/1271-014-A, August 8, 1993, 93/0029)

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. On gestation day 13, females were euthanatized and inspected for pregnancy by cesarean section. Viable fetuses, early and late resorptions and deaths were counted. Results: the only treatment related effect was salivation immediately following dosing at the high dose. There were no adverse effects on male, fertility, reproductive performance, or sperm count or mortality and there were no pathological changes in the male reproductive organs.

Comments: 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.

3. A study of the effects of FTC on embryo/fetal development in mice, Lot # 3821A, December 30, 1998, (TOX-037, 345003/Document No. 3566)*

Groups of presumed pregnant female mice (strain: Crl: CD-1 (ICR)BR; 25 dams/group; total animals: 120) were administered FTC via oral gavage twice daily (approximately 6-hr between doses) at a total dose levels of 0 (vehicle control), 250 (low), 500 (mid) or 1000 mg/kg/day (high) during gestation days 6 through 15 to study the potential maternal and developmental toxicity of FTC. Dams (5/group) in the toxicokinetic arm for each dose level were treated similarly except that on gestation day 15 following a single dose of 250, 500 or 1000 mg/kg, they were exsanguinated at 0.5 hr post dose and plasma was harvested for toxicokinetic evaluation. Results: all maternal animals survived to the scheduled necropsy on gestation day 18; no treatment-related internal findings were noted at any dose level. No clinical signs of toxicity were observed in any treated group. Mean maternal body weight gain and food consumption were unaffected by treatment at all dose levels. Intrauterine growth and survival were not affected by the test article administration at any dose level and there were no external, visceral or skeletal malformations or developmental variations observed at any dose. No treatment-related malformations or developmental variations were observed at any dose level. Toxicokinetic blood samples had mean plasma FTC concentrations of 52 ± 15; 88 ± 14 and 186 ± 14 µg/ml for the low, mid and high dose levels, respectively.

Comments: 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.

4. Toxicokinetic study to determine fetal exposures in CD-1 mice given FTC orally (gavage), November 3, 2000 (TOX103/ —— 345005)

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 (divided dose with 6 hr between doses) between gestation days 6-14 in a toxicokinetic study to determine fetal exposures. On gestation day 15, animals were sacrificed, maternal plasma was harvested and the fetuses were removed, pooled within litters and homogenized in 1 volume (w:v) of normal saline. Concentrations of FTC were determined using a validated —— method. Results: all animals survived to gestation day 15. One female (high) was found to be nongravid and this animal was removed from the study. 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.

5. A study of the effects on embryo/fetal development in rabbits, lot # TP-0006/96/DDD, June 16, 2000, (TOX038/ -345004)*

Groups of artificially inseminated New Zealand White rabbits (20 animals/group + toxicokinetic phase 5 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 (approximately six hr apart) from gestation days 7 through 19 to evaluate the potential maternal and developmental toxicity of FTC. Maternal and fetal blood samples were obtained at appropriate intervals and plasma samples were analyzed for concentrations of FTC using a validated —— method. Results: one female (mid) was found dead on gestation day 20 due to an intubation error during the dose administration period. One female (each control and low) died on gestation days 20 and 25, respectively. One female (high) aborted on gestation day 23 (this abortion was considered by the sponsor to be spontaneous in origin based on the frequency of abortion in the historical control data-base). All other females survived to the scheduled necropsy on gestation day 29; no treatment related maternal findings were noted at any dose level. The only drug related clinical sign observed was decreased defecation in animals (high). Body weight gain and food consumptions were reduced (< 10%, $p<0.05$ or 0.01) in the mid (gestation days 15-20) and high (11-15) dosed animals. Intrauterine growth and survival were unaffected at any dose level. No treatment related malformation or developmental variations were observed in fetuses at any dose level. Pharmacokinetic estimates are summarized in Table 1.

Table 1
Mean pharmacokinetic parameters of FTC in maternal rabbits

Dose level (mg/kg/day)	Cmax (µg/ml)	AUC_{0-24} (hr*µg/ml)	Tmax (hr)	Fetal/maternal ratio*
100	16	87.3	1	0.42
300	44.2	315.2	1.4	0.51
1000	143.3	1257.8	1.7	0.41

*=estimated at 1-hr post dose on gestational day 20

Comments: In this study, no maternal toxicity was apparent at a dose level of 100 mg/kg/day. No developmental toxicity was observed at any dose level. Based on the results of this study, 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).

6. FTC: Pre- and postnatal development in CD-1 mice (Segment III), Lot # TP-0006/96/DD, Triangle Pharmaceuticals, Durham, NC, March 11, 1999, (TOX-039/Document No. 5063)*

Groups of presumed pregnant female mice (strain: Crl: CD-1 (ICR)BR; 25 dams/group; weight: 30-30 g) 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 to produce alterations in pre- and postnatal growth, development, parturition and lactation in both the F_1 and F_2 generations. The F_0 dams raised their pups through weaning, at which time the F_1 weanlings were allowed to mature to at least 10 weeks of age. F_1 animals were then cohabited within dose groups

and the resulting F_2 litters were evaluated and the pups were sacrificed. Results: no F_0 maternal toxicity occurred, including no mortalities, no effects on maternal body weight or weight change and no decrease for feed consumption were seen during gestation. During lactation, 4 F_0 dams died (2 each in low and high). The cause of death was not determined. Two entire litters were found dead (1 each in controls and low). There were no significant effects on maternal lactation, body weighs, weight changes or feed consumption in any dose group. There were no treatment-related effects on any reproductive or lactational indices, nor were there any significant changes in F_1 litter size, pup body weights or sex ratio across groups. There were no treatment-related findings for pups which died or were euthanized moribund. There were no effects of treatment on the acquisition of vaginal patency or preputial separation. Neither motor activity nor pupillary reflex were significantly affected for any treatment group. Necropsy of F_0 dams indicated no significant findings. During the postweaning period, the body weights of F_1 male and female mice were essentially equivalent across all groups. The estrous cycles was longer by an average of 0.64 days ($p<0.01$) in F_1 females (high). However, estrous cycles were otherwise normal at all doses and essentially all females in all groups were cycling. During the gestation of F_1 dams (to produce F_2 litters), there were no effects on maternal body weights, weight changes or clinical observations. There were no effects on maternal body weights, weight changes or clinical observations. There were no effects on F_1 male or female reproductive indices or on F_1 female gestational or lactational indices. F_2 pup survival indices, body weights and sex ratios were not affected.

Comments: 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 under the conditions of the study.

IX. SPECIAL TOXICOLOGY STUDIES

None reported

X. CONCLUSIONS AND RECOMMENDATIONS

In the clinic, the test compound is being administered as an oral formulation (200 mg capsule). The human $AUC_{0-\infty}$ (at steady state) at the dose of 200 mg/day is approximately 10.0 µg*hr/ml. The kinetic data from subchronic/chronic toxicology studies in animals showed that the mean AUC values achieved in the animal studies at the NOELs/NOAELs were several fold higher than that of the therapeutic dose in humans (Appendix # 2). 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).

Emtricitabine can be classified as Pregnancy Category B.

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 and two-year carcinogenicity studies in mice and rats are in progress.

The issue of labeling will be reviewed separately.

XI: APPENDIX

1. Synopsis of FTC single dose (acute) acute animal toxicity studies.

Table 1
Summary of FTC single dose (acute) toxicity studies

Species	Dose level (mg/kg)	Results	Tolerated dose (mg/kg)	BSA equiv. in humans per day
Mice	700 iv	There were no deaths, no clinical sign of toxicity and no abnormal findings.	700	3.4 g
Mice	1050 po		1050	5.1 g
Rat Wistar	1400 po		1400	13.6 g
CD-1 mice	4000 po	There were no deaths, no clinical symptoms, no drug related changes and no gross pathological findings.	4000	19.4 g
CD-1 mice	200 iv	There were no deaths, no clinical symptoms, no drug related changes and no gross pathological findings.	200	973 mg
CD rats	4000 po	There were no deaths, no clinical symptoms, no drug related changes and no gross pathological findings.	4000	38.9 g
CD rats	200 iv	There were no deaths, no clinical symptoms, no drug related changes and no gross pathological findings.	200	1.9 g

BSA = Body surface area equivalent factor

2. Synopsis of FTC subchronic/chronic oral toxicology studies in animals and comparison with the clinical doses.

Table 1
Summary of FTC subchronic/chronic oral toxicity studies in animals and comparison with the clinical dose (200 mg dose, AUC = 10 µg*hr/ml)

Animal study	Dose (mg/kg/day	Results & NOEL/NOAEL	AUC_{0-24h} (µg*hr/ml)	Safety margin relative to human AUC
1-month mice	120		85	
	600	600	385	38.5X
	3000	Mild anemia	1740	
6-month mice	167		88	
	500		350	
	1500	1500	1430	143X
6-month mice	120		143	
	600	600	538	53.8X
	3000	Mild anemia, ↑ urinary volume	1787	
3-month rats	120		67	
	600		346	
	3000	3000	1461	146X
1-month monkeys	80		70	
	400	400	350	35X
	2000	Soft feces in females	1430	
3-month monkeys	40		27	
	200		121	
	1000	1000	621	62X
12-month monkeys	50		21	
	200	200	98	9.8X
	500	↑ MCH, ↓ RBC counts	273	

3. Synopsis of FTC animal reproductive toxicology studies

Table 1

Summary of FTC reproductive toxicology studies. The clinical dose is 200 mg/day, AUC = 10 µg*hr/ml

Animal study	Dose (mg/kg/day	Results & NOEL/NOAEL (mg/kg/day)	BSA equiv. in humans
Seg I, mice:	250	No effects were observed at any dose.	

fertility & embryonic development	500 1000	NOAEL: 1000 for reproductive and developmental toxicity	4.8 g
Seg I, rats: male fertility	150 750 3000	No effects were observed at low or mid dose. Post dosing salivation at high dose NOAEL: 3000 for male fertility or early fetal development in naive females	26.2 g
Seg II, mice: developmental	250 500 1000	No effects were observed at any dose. NOAEL: 1000 for teratogenic, embryotoxic or maternal effects	4.8 g
Seg II, rabbits: developmental	100 300 1000	Low: no observed effects, AUC=87.3 µg*hr/ml Mid: body weight gain & feed consumption reduced; AUC=315.2 µg*hr/ml High: body weight gain & feed consumption reduced; AUC=1257.8 µg*hr/ml NOAEL: 100 for maternal toxicity NOAEL: 1000 for teratogenic effects	*8.7X *125.7X
Seg III, mice: pre & postnatal developmental	250 500 1000	No effects were observed at low or mid dose. High: F1 dams had estrous cycles slightly longer (0.6 day) than controls. NOAEL: 1000 for reproductive and postnatal toxicity	4.8 g

BSA = Body surface area equivalent factor
*=comparison of animal AUCs with that of clinical dose

4. Synopsis of single oral dose pharmacokinetic parameters of FTC in animals

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

Species	Dose (mg/kg)& route	Pharmacokinetic parameters					
		AUC (µg*hr/ml	Cmax (µg/ml)	Tmax (min)	Vdss (l/kg)	Cl (l/hr/kg	F (%)
Mice	600 iv	473	-	-	1.1	1.3	-

	600 po	296	139	40	-	-	62.7
Mice	100 iv	44.7	-	-	0.94	2.2	
	100 po	35.3	22	24.5	-	-	79
Mice	10 iv	4.3	-	-	0.89	2.3	-
	10 po	4.1	2.4	25.4	-	-	96
Monkeys	80 iv	86.1	-	-	0.77	0.97	-
	80 po	83.6	39.4	53	-	-	97.4
Monkeys	10 iv	14.8	-	-	0.8	0.7	-
	10 po	9.2	3.5	78	-	-	62.7
Monkeys	200 po	133	46.7	60	-	-	-

Table 2

Disposition of radioactivity after a single oral administration of radioactive FTC in animals. All values are presented as mean values

Species	Dose (mg/kg)	Plasma		Recovery (% dose)			Metabolites (% dose)	
		Tmax (hr)	Cmax (µg/ml)	Total	urine	feces	urine	feces
Mice	120, ^{3}H	nd	nd	85	66.8	18.1	9	<1
Rats	200, ^{14}C	52	1	98.6	79.3	18.4	7	4
Monkeys	200, ^{14}C	54.3	1	84.5	40.8	35.4	11.6	1.3
Monkeys	80, ^{3}H	nd	nd	84	41	33	36	2

nd= not done

*= mouse: 5-fluorocytosine and 3-sulfoxides
rat: not done
monkeys: 3-sulfoxides, 2-glucuronide, 5-fluorocytosine and 2-glucuronidated and deaminated metabolites mainly detected in gut, gut contents, kidney and liver

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5. Synopsis of multiple oral dose pharmacokinetic parameters of FTC in animals

Table 1
Mean pharmacokinetic parameters of FTC following 6-month of oral gavage toxicity study in mice

Dose (mg/kg/day)		3-month		6-month	
		Cmax (µg/ml)	AUC_{0-t} (hr*µg/ml)	Cmax (µg/ml)	AUC_{0-t} (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

Table 2

Mean pharmacokinetic parameters of FTC following 6-month of oral gavage toxicity study in rats

Dose (mg/kg/day) & Sex		C_{max} (µg/ml)		Tmax (hr)		T½ (hr)		AUC_{0-24} (µg*hr/ml)	
		Day 2	day 90	day 2	day 90	day 2	day 90	day 2	day 90
120	♀	18.0	21	1	1	2.36	6.5	55.5	69
	♂	15.3	17.5	1	1	2.41	5.2	61.63	66
600	♀	73.1	120	1	1	2.38	3.5	260.37	362
	♂	59.2	78	1	1	3.58	3.8	295.96	329
3000	♀	225	323	2	2	2.67	4.5	1357	1646
	♂	211	222	2	2	3.8	4.5	1413	1276

Table 3

Mean pharmacokinetic parameters of FTC during 52-week of dosing in monkeys

Group; dosage (mg/kg/day)		AUC_{0-24} (µg*hr/ml)				Cmax (µg/ml)			
		drug weeks				drug weeks			
		Day 0	13	26	52	day 0	13	26	52
low 50	♂	-	16.1	17	18.1	3.6	3.7	3.7	4.3
	♀	-	18.1	22.9	33.3	1.5	2.9	4.8	7.3
mid 200	♂	-	75	103	97	12.1	11.1	15.6	17.4
	♀	-	100	120	98	14.2	16.9	24.8	21.3
high 500	♂	-	266	342	274	31.6	42	51	51
	♀	-	196	321	238	37	27	39	38

- : not available

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