CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-527

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-527
Submission date: December 21, 2009
Drug: GILENYA (fingolimod)
Applicant: Novartis Pharmaceuticals
Indication: treatment of adults with relapsing multiple sclerosis (MS) to reduce the frequency of clinical exacerbations and to delay the accumulation of physical disability

Reviewing Division: Division of Neurology Products

Introductory Comments:
The primary pharmacology/toxicology reviewer recommended that this NDA not be approved from a pharm/tox perspective based primarily on toxicity findings observed in lungs in multiple species. The supervisor considered these concerns in a secondary review and concluded that the application could be approved from a pharm/tox perspective. The primary review did not recommend any particular additional nonclinical studies. The supervisor recommended that the applicant conduct a juvenile animal study in rat as a post-marketing requirement.

General toxicity:
Toxicity to the lung in multiple species (mouse, rat, dog, monkey) included findings such as bronchio-alveolar hyperplasia, inflammation, smooth muscle hypertrophy and pleural thickening. The toxicity was also manifest at necropsy as a failure of the lungs to collapse, as is normal. The lung findings are described in more detail in the primary and secondary reviews. The NOAELs for the lung toxicity in rat and dog were below the human dose when expressed on a mg/m^2 basis. The exact mechanism for the injury is not clear, although the applicant did conduct some studies suggesting that drug-induced bronchoconstriction may play a role. The sponsor did include some assessments of lung function in clinical studies.

Reproductive and developmental toxicity:
The primary pharm/tox reviewer and supervisor have detailed the reproductive and developmental toxicity studies of fingolimod in their reviews. These studies show that fingolimod produced some fetal malformations and deaths in rats at doses below the human dose on a mg/m^2 basis. Fetal death and growth retardation were observed in rabbits, although at doses higher than the recommended human dose.

Pup survival was decreased and several adverse effects were noted in neurobehavioral development endpoints in a rat pre-postnatal study. The neurobehavioral effects were noted at a dose several fold higher than the human dose on a mg/m^2 basis.

Carcinogenicity:
Lifetime carcinogenicity studies were conducted in mice and rats. The Executive Carcinogenicity Assessment Committee concluded that fingolimod produced drug-related

increases in malignant lymphomas in both male and female mice at the mid and high doses (0.25 and 2.5 mg/kg/day). No drug-related neoplasms were observed in rats. There was some question about the quality of the mouse study because a number of tissue samples were described as autolyzed. The supervisor has considered this in her memo and concluded that although the autolysis was undesirable it does not appear to have prevented the study pathologists from assessing tissues for tumors and that, therefore, the assessment was acceptable.

Conclusions:
The lung toxicity observed in the nonclinical studies is of some concern because it is reproducible across several species and occurs in some cases at doses below those used in humans. This toxicity was apparently identified early enough in the development of fingolimod to permit assessment of lung toxicity in clinical trials. Therefore, I agree with the pharm/tox supervisor that this NDA may be approved from the pharm/tox perspective if the clinical data are adequate to address this toxicity.

The pharm/tox supervisor has recommended that the applicant conduct a juvenile animal toxicity study in rats to support pediatric clinical studies and possible approval in the pediatric population. Some neurobehavioral effects were noted in the pre-postnatal study in rats. The impact of fingolimod on reproductive function and neurobehavioral development has not been fully assessed in a juvenile animal model. I agree that such a study can be conducted as a post-marketing requirement.

I agree with the labeling suggestions outlined in the supervisory pharm/tox memo.

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/s/

PAUL C BROWN
09/21/2010

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: September 20, 2010

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-527 (Gilenya®, fingolimod, FTY720), received December 21, 2009

Novartis Pharmaceuticals has submitted NDA 22-527 to support marketing approval for Gilenya (FTY720, fingolimod) for treatment of adults with relapsing multiple sclerosis (MS) to reduce the frequency of clinical exacerbations and to delay the accumulation of physical disability. NDA 22-527 was submitted as a rolling NDA, with the final submission (Module 5 Clinical Reports) received on December 21, 2009. To support this application for treatment of MS, the sponsor has conducted a complete battery of nonclinical studies, which have been reviewed in detail by Dr. Siarey (*Pharmacology/Toxicology NDA Review and Evaluation NDA 22-527, Richard Siarey, Ph.D., September 20, 2010).* Based on his review, Dr. Siarey recommends that the NDA not be approved, primarily due to lung toxicities observed in multiple animal species. In addition, Dr. Siarey notes several nonclinical deficiencies:

- The reproductive and developmental toxicity studies are inadequate due to lack of fetal assessment at all doses.
- For the *in vitro* genetic toxicology studies, there is a lack of data on the amount of FTY720-P formed by the metabolic activation system used (rat liver S9).
- The mouse carcinogenicity study is inadequate due to the high rates of autolysis reported.
- There are insufficient safety data for certain metabolites, for which circulating levels increase substantially in special patient populations (i.e., renal impairment).

This memo will briefly summarize the nonclinical findings, with more detailed discussion of specific target organ toxicities, and will address the nonclinical deficiencies noted by Dr. Siarey.

- **Pharmacology**

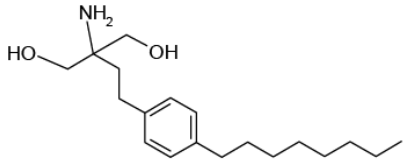
FTY720 (fingolimod) is a sphingosine 1-phosphate (S1P) modulator "…derived from ISP-1 (myriocin), a fungal metabolite [of the ascomycete *Isaria sinclairii*] that is an eternal youth nostrum in traditional Chinese herbal medicine…" (Brinkmann V *et al*. *J Biol Chem* 277(24):21453-21457, 2002). FTY720 itself has essentially no activity at S1P receptors, but when phosphorylated to FTY720-P (the S-enantiomer), exhibits high *in vitro* binding affinity at four of the five subtypes of the S1P receptor (EC_{50}'s of 0.3, 3.1, 0.6, and 0.3 nM for $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$, respectively; EC_{50} for $S1P_2$ was >10000 nM). FTY720-P demonstrates both agonist and antagonist effects at S1P receptors. Acute binding of FTY720-P to S1P receptors results in agonist effects, as assessed in agonist-induced GTY[γ-35S] binding assays and other *in vitro* functional assays (e.g., endothelial cell preparations). However, with time and increased duration of dosing, FTY720-P causes SIP receptor internalization and degradation, thus leading to functional antagonist effects. Pharmacological activity of FTY720-P at the $S1P_1$ receptor is thought to underlie its efficacy in patients with MS. *In vivo,* FTY720-P binds to SIP receptors on lymphocytes, inhibiting egress of $CD^{+4}CD^{+8}$ T-cells and B-cells from peripheral lymphoid organs, resulting in a dose-related decrease in circulating lymphocytes and entry of autoreactive T-cells into brain. FTY720 exhibited efficacy in a number of animal models of MS (e.g., myelin basic protein-induced EAE in Lewis rat, proteolipid protein-induced EAE in SJL/J mice, myelin oligodendrocyte glycoprotein-induced EAE in C57BL/6 mice).

Activity at other S1P receptor subtypes, as well as at $S1P_1$, may be involved in some of the adverse effects observed in animals and/or human. For example, S1P receptors have been demonstrated to have a critical role in development (Allende ML, Proia RL *Biochim Biophys Acta* 1582:222-227, 2002; Chae S-S *et al*. *Develop Biol* 268:441-447, 2004; Kono M *et al*. *J Biol Chem* 279(28):29367-29373, 2004; Mizugishi K *et al*. *Mole Cell Biol* 25(24):11113-11121, 2005), in cardiovascular function (e.g., Forrest M *et al*. *J Pharmacol Exp Therap* 309:758-768, 2004), and in pulmonary integrity (Gon Y *et al*. *PNAS* 102(26):9270-9275, 2005).

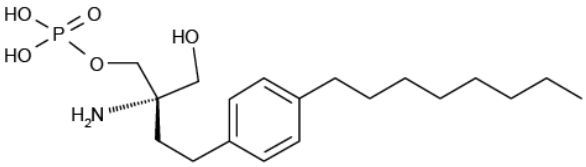
- **Metabolism**

In all animal species tested (and human), FTY720 is phosphorylated to the active moiety, the S-enantiomer of FTP720-P. In humans, the ratio of FTY720-P to FTY720 is ≈0.5 at steady state (0.5 mg/day). In addition to FTY720-P, the primary metabolites in humans are M2 (hexanoic acid metabolite), M3 (butanoic acid metabolite), and M30 (C18-ceramide metabolite) (structures provided below; from sponsor).

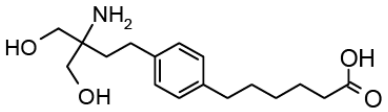
FTY720



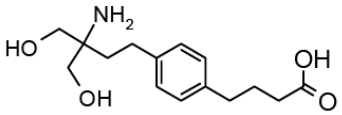
(S)-FTY720-P



M2



M3



The sponsor characterizes M2 and M3 as inactive; however, it is unclear what this conclusion is based on. A search of nonclinical summaries, and what would appear to be relevant study reports, did not identify any data on the pharmacological activity of either metabolite.

After an acute dose in non-renally impaired subjects/patients, M2 and M3 account for <10% of total drug-related material (8.9 and 8.3%, respectively; FTY720-P accounts for 23.3%), i.e., neither is a major circulating metabolite. However, in severely renally-impaired patients, circulating levels (AUC) of these metabolites (as well as FTY720 and FTY720-P) are increased (300 and 1350%, respectively) (cf. *Clinical Pharmacology/Biopharmaceutics Review, NDA 22-527, Ju-Ping Lai, Ph.D., Jagan Parepally, Ph.D, 7/21/2010*).

POPULATION	M2		M3	
	C_{max} (ng/mL)	AUC (ng*hr/mL)	C_{max} (ng/mL)	AUC (ng*hr/mL)
severe renal impairment	0.844	109.440	5.082	1002.995
control	0.639	76.723	0.631	73.979

Therefore, in these patients, plasma exposure (AUC) for M3 and possibly for M2 increases sufficiently so that both would be expected to meet the criterion for a major circulating metabolite (i.e., ≥10% of total drug-related material).

The available nonclinical TK data (provided below; expressed as means for male-female) indicate that M2 and M3 have been adequate assessed in most of the definitive nonclinical studies, with the clear exceptions being the pre- and post-natal development and carcinogenicity studies in rat. In the fertility and early embryonic development and embryo-fetal development studies in rat, the HD was 10 mg/kg; therefore, M2 and M3 were reasonably assessed in those studies. However, the HD used in the pre- and post-natal development study was only 0.5 mg/kg due to increased perinatal mortality at the HD. The mouse and rat carcinogenicity studies were conducted at lower doses than used in the subchronic or chronic toxicity studies (HD of 2.5 mg/kg in both species); metabolites M2 and M3 were not analyzed in either of the carcinogenicity studies. Based

on the 26-wk data in rat, plasma AUCs for M2 and M3 at the HD used in the rat carcinogenicity study would be expected to have been at least 3-fold lower than that in renally impaired humans at the recommended clinical dose. There are no definitive steady-state plasma data in mouse for either M2 or M3 at any dose; however, based on the acute-dose PK data, it is likely that plasma AUCs achieved for these metabolites in the mouse carcinogenicity study would have been at least similar to those in seen in humans with severe renal impairment.

STUDY	DOSE (mg/kg)	SAMPLING TIME	AUC	
			M2	M3
26-wk rat (#991052)	7.5 (HD)	Wk 22	66.7-42.0	1170-1050
39-wk monkey (#997040)	3 (HD)	Wk 40	328-366	899-1099
acute dose mouse (#R0100826)	2.5	acute	40	4620
13-wk mouse*	5 (HD)	--	25	2633

*estimated by sponsor from acute dose (2.5 mg/kg p.o.) PK study data (Study R0100826)

Dr. Siarey notes that "There is a lack of coverage for metabolites in patients with impaired function of certain organs, such as kidneys…" The Clinical Pharmacology team has recommended that the sponsor conduct nonclinical studies to assess the toxicity of M2 and M3 to support administration to renally impaired patients. However, considering all the available nonclinical data and assuming that the data in severely renally impaired patients provide an adequate characterization of plasma exposure to M2 and M3, it is my opinion that no additional studies are needed for either metabolite.

- **Nonclinical findings**

General Toxicity: definitive oral toxicity studies of up to 26, 26, and 52 weeks duration were conducted in Sprague-Dawley or Han:Wistar rat, Beagle dog, and cynomolgus monkey, respectively. A number of target organs were identified in these studies: lymphoid tissue (including lymph nodes, thymus, spleen), male reproductive organs (prostate, epididymides, seminal vesicles, testes), pancreas, kidney, heart, vasculature, brain, peripheral nerves, liver, adrenal gland, and lung. Findings in these organs are described in detail in Dr. Siarey's review. Some adverse effects were marked (e.g., degeneration of heart nerve fibers and Auerbach's plexus, axonal and Schwann cell degeneration in nerve fiber bundles in the coronary sulcus of the heart, adrenal medullary degeneration/fibrosis), but were observed primarily at high doses. Findings in heart were observed in rat (primarily in Wistar rat; proposed by the sponsor to be the result of strain-specific susceptibility to drug-induced vasculopathy), dog (wall thickening, proposed by the sponsor to be secondary to hemodynamic changes), and monkey. In the 52-wk monkey study, microscopic changes in heart were observed at all doses (1-10 mg/kg), including myocardial hypertrophy, arterial mural thickening, and endocardial fibrosis. However, similar effects were not observed in monkey at doses of 0.5 or 3 mg/kg given for 39 weeks.

The most sensitive target organs, consistently affected in all species, were lymphoid tissue and lung. Effects on lymphoid tissue (atrophy) were considered to reflect the pharmacodynamic activity of FTY720 (i.e., down-regulation of S1P receptors on

lymphocytes). Microscopic findings reported for lung are summarized in the attached tables. In addition to these findings, it is notable that in all species there were instances reported in which lung(s) was not collapsed upon necropsy. In rat, a small increase in this finding was observed at the HD in the 2-year carcinogenicity study; the highest no-effect dose in that study (0.5 mg/kg) is ≈10 times the recommended clinical dose of 0.5 mg/day, based on mg/m^2. In monkey (39- and 52-wk studies, but not in 2- or 13-wk studies), insufficient lung collapse occurred at all doses and was correlated with smooth muscle hypertrophy (an effect observed in all species) and/or collagen deposition; no no-effect dose was established in the 39-wk study. In addition, in the 52-wk monkey study, respiratory distress was observed in treated (3 and 10 mg/kg, but not 1 mg/kg) animals sedated with ketamine. The sponsor described observations as follows:

> In Week 26, Monkeys 657M, 659M, 658F & 664F (10 mg/kg/day) had apparently normal respiration prior to Ketamine sedation for ophthalmoscopy but developed very laboured respiration when sedated. Respiratory distress is not normally associated with Ketamine sedation, therefore this was considered to be treatment-related. Three of the animals recovered within about 15 to 20 minutes (as sedation regressed), however Animal 657M was more severely affected. This monkey experienced considerable respiratory distress and hypoxia/blue appearance developed. The animal was placed in an incubator and oxygenated. Profuse salivation developed (not included in the incidence summary). In order to ensure that this salivation did not interfere with breathing, 0.15 ml Atropine was injected by the intramuscular route to reduce salivation. The animal fully recovered within about 30 minutes and was returned to its cage. In order to minimise the possibility of similar adverse reactions in Week 52 compromising survival (and thus the integrity of the study) pre-terminal ophthalmic examinations for animals receiving 10 mg/kg/day were re-scheduled for Week 53, whilst under Ketamine sedation prior to necropsy.
>
> Animal 657M (10 mg/kg/day) again exhibited respiratory distress during blood sampling procedures in Week 52. Whilst restrained on its dorsal surface for collection of blood from the femoral vein, Animal 657M experienced difficulty in breathing. The animal was salivating at the time and it was speculated that the respiratory distress may have been partially associated with inhalation of saliva, (although this animal had previously being demonstrated as prone to exhibiting respiratory problems under sedation for ophthalmoscopy during Week 26). It was necessary to administer oxygen to aid recovery and, as a consequence, the animal was dosed approximately one hour late that day.
>
> During Week 53 whist under Ketamine sedation immediately prior to necropsy, Monkeys 657M, 660F and 662M (10 mg/kg/day) and Monkey 654F (3 mg/kg/day) all exhibited laboured breathing.
>
> These incidences of respiratory distress are considered to be associated with treatment, as evidenced by the pathological changes in the lungs (see **TERMINAL STUDIES**).

As the sponsor points out, respiratory distress or depression is not generally associated with ketamine anesthesia. Ketamine tends to increase blood pressure and heart rate, and suppresses respiration less than other anesthetics. The sponsor further assessed this finding in a mechanistic study in male Wistar rats (#PKF-99-00746). An acute oral dose (10 mg/kg) of FTY720 resulted in significant airway obstruction, with the effect being maximal on Day 3 post dose. In combination with ketamine, FTY720 at doses of 1 and 10 mg/kg resulted in increased bronchoconstriction; 1 mg/kg FTY720 alone had no effect. In discussing the potential biological relevance of the finding, the sponsor concluded that "A similar degree of airway obstruction in patients without other airway

diseases would certainly not be life-threatening or even damaging, however it would most likely be conceived as unpleasant." The sponsor proposed two possible mechanisms: a direct bronchoconstrictor effect of FTY720 (acute), which "maintained over several days" leading to smooth muscle hypertrophy, which would "…clearly impair lung mechanics as it reduces lung elasticity." Other mechanistic studies conducted by the sponsor also demonstrate bronchoconstrictive effects of FTY720.

In a recent published study, FTY720 (0.5 mg/kg i.p. 3x/wk for 2 weeks) was reported to exacerbate antibiotic (bleomycin)-induced lung injury (e.g., collagen deposition, fibrosis) in adult male C57BL/6 mice and decreased survival (Shea BS *et al*. *Am J Respir Cell Mol Biol* 2010 Jan 15 [Epub ahead of print]).

Dr. Siarey bases his NA recommendation primarily on the lung toxicity observed in animals with FTY720. While the nonclinical data clearly indicate the potential for FTY720 to induce substantial lung toxicity, this toxicity was evident early in the development of FTY720 and was monitored for in clinical trials. The clinical data indicate dose-related decreases in pulmonary function parameters (forced expiratory volume over one second [FEV1] and diffusion lung capacity of carbon monoxide [DLCO]), including at the recommended clinical dose of 0.5 mg/day. These data are described in labeling, which includes a recommendation for evaluation of respiratory function in patients taking GILENYA "…if clinically indicated." Therefore, it is my opinion that the nonclinical findings are not a reason not to approve the NDA. However, there is a concern that longer duration of therapy, or use in patients with asymptomatic pulmonary abnormalities or in combination with other drugs affecting pulmonary function, might result in additional adverse effects. For this reason, I would recommend that the nonclinical lung findings be described in labeling in Section 13.2 Animal Toxicology.

Reproductive and Developmental Toxicology: the sponsor conducted a complete battery of oral reproductive and developmental toxicology studies.

Fertility and early embryonic development: the effects of FTY720 on mating and fertility were assessed in male and female Sprague-Dawley rats at oral doses of 0, 1, 3, and 10 mg/kg. Males were dosed for only 28 days prior to mating. The sponsor justified this treatment period based on a lack of testicular toxicity in two 1-month toxicity studies. (In this study, atrophy of the prostate and seminal vesicle was observed in 2 HDM; mating of one of these males did not result in a pregnancy.) Females were dosed for two weeks prior to mating. Both males and females were dosed during the 10-day mating period. Dosing continued in males until the day before sacrifice and in females, if pregnant, until GD 7. Females that had not successfully copulated were "dosed until the day prior to the last day of the mating period".

The only dose-related effect was a slight decrease in the number of pregnant females at the HD (89% vs 94-100% in the other groups).

Embryo-fetal development: two definitive embryo-fetal studies were conducted in rat and one in rabbit.

Rat: in the first study, FTY720 was administered at doses of 0, 1, 3, and 10 mg/kg. The number of fetuses was reduced at all doses, due to early and late resorptions; there were only 17 live fetuses at the MD and no live fetuses at the HD. FTY720 was clearly teratogenic, with increases in external (edema) and visceral (including ventricular septal defect and persistent truncus arteriosus) malformations at both lower doses. A second study of FTY720 was conducted at doses of 0, 0.03, 0.1, and 0.3 mg/kg in an attempt to establish a no-effect dose for teratogenicity. (Skeletal examination was not conducted at the lower doses in this study.) There was no increase in external malformations, but the incidence of visceral malformations (including persistent truncus arteriosus and ventricular septal defect) was increased at the MD and HD, and possibly the LD. The only finding at the LD was an increased incidence of left umbilical artery. This finding was increased at all doses, but the effect was statistically significant only at the LD. It is difficult, however, to dismiss this as a drug-related effect since, in the previous study, the incidence of left umbilical artery was dose-dependently increased at 1 and 3 mg/kg. Left umbilical artery was detected in only one control fetus in the original study, and in no control fetus in the lower-dose study. Adverse effects of FTY720 on vascular development are consistent with the role of $S1P_1$ in vascular maturation (Allende ML *et al*. *Blood* doi:10.1182/blood-2003-02-0460; Chae S-S *et al*. *Develop Biol* 258:441-447, 2004).

Rabbit: two dose-ranging studies were conducted in order to select doses for the definitive study. In the first dose-ranging study, FTY720 was administered at doses of 0, 0.01, 0.03, 0.3, and 3 mg/kg; no adverse effects on dams or fetuses were observed. The second dose-ranging study was conducted using higher doses (0, 10, 30, and 60 mg/kg). Abortions occurred at all doses, and post-implantation loss was increased at all doses (≈62% at the LD), with 100% post-implantation loss at the HD. There were no live fetuses at the MD or HD, and the number of live fetuses was significantly reduced at the LD. Therefore, doses of 0, 0.5, 1.5, and 5 mg/kg were selected for the definitive study.

In the definitive study, increases in early and late resorptions occurred at the MD and HD, resulting in a significant decrease in the number of live fetuses at the HD. While there was a statistically significant increase in the number of fetuses with skeletal malformation at the HD, only two HD litters were affected. Therefore, there is not a clear signal for teratogenicity in rabbit.

Pre- and post-natal development (including maternal function): the effects of FTY720 on pre- and post-natal development were tested in rat at doses of 0, 0.05, 0.15, and 0.5 mg/kg. High-dose selection was based on findings in the embryo-fetal studies in rat, including the increased incidence of visceral malformations at doses ≥0.1 mg/kg. There was no evidence of maternal toxicity at any dose. Findings in the F_1 generation include an increase in stillborn pups at the HD (7 pups from 6 litters), and decreases in postnatal survival at all doses (primarily during the first week postpartum). The total number of pups surviving to the end of weaning was significantly decreased at all doses. Attainment

of developmental landmarks did not appear adversely affected. Open-field motor activity was reduced in MDF and HDF. Adverse effects on neurobehavioral development were observed on the passive avoidance task in HDM (89.5% reached criterion vs 100% of pups in other groups) and on the water M-maze in HDF (90% reached criterion vs 100% of pups in other groups). Regarding reproductive performance of the F_1 generation, the only effect was a small, but dose-related decrease in the fertility index (100, 95.8, 88, and 87% in C, LD, MD, and HD, respectively).

Dr. Siarey notes certain deficiencies in the reproductive and developmental toxicology assessment, specifically (1) the lack of sufficient MDF in the fertility study and (2) the lack of fetal skeletal examination at the LD and MD in the low-dose embryo-fetal development study in rat. While Dr. Siarey is correct in noting these deficiencies, it is my opinion that neither compromises the respective study to an extent that would require additional nonclinical studies. Regarding the fertility study, although there was less than the recommended number of MDF, there were an adequate number of females (16) at the HD. Regarding the low-dose embryo-fetal development study in rat, the sponsor elected not to conduct fetal skeletal examinations at the lower doses because of a lack of effect at the HD. Assessment of only C and HD fetuses is generally not acceptable, although the ICH S5 guidance states, "The examination of mid- and low-dose fetuses for visceral and/or skeletal abnormalities may not be necessary where the evaluation of the high-dose and the control groups did not reveal any relevant differences". The OECD guideline for prenatal developmental toxicity studies (414, 22nd January, 2001), CFSAN's Redbook, and a recent industry publication on the design of embryo-fetal studies (Wise LD *et al*. *Birth Defects Res (Part B)* 86:418-428, 2009) recommend fetal evaluation in all groups. A major concern regarding examination of only C and HD fetuses is that a drug-related, but not necessarily dose-related, finding may be missed, particularly considering how rare many malformations are. In this case, however, FTY720 was clearly teratogenic at the MD used in the low-dose embryo-fetal study, and possibly teratogenic at the LD. In addition, the LD is almost 2-fold lower than the recommended clinical dose of 0.5 mg/day0, on a mg/m^2 basis. Therefore, the need for skeletal data at the lower doses is not critical since there is no safety margin for teratogenicity in the rat.

Carcinogenicity: the carcinogenic potential of FTY720 was assessed in 104-wk oral studies in CD-1 mouse and Han:Wistar rat.

Concerns regarding the validity of both studies were raised by the Division of Special Pathogen and Immunologic Drug Products (DSPIDP; currently, the Division of Special Pathogen and Transplant Products, DSPTP) when the studies were submitted to IND 57293 (March 17, 2005). Dr. Siarey has discussed the regulatory history of this concern in his review. Briefly:

- On May 12, 2005, the sponsor was asked to address questions regarding "the number of animals with autolytic tissues, and inconsistent diagnoses throughout the submission" in one of two ways: (1) submit tissue slides to the FDA for examination or (2) obtain independent peer review of "…all tissues of all animals in both studies"; the sponsor elected to conduct an independent peer review (*cf. Memorandum of Telecon, May 12, 2005; filed November 29, 2005*).

- On July 13, 2005, the sponsor was told by Dr. Terry Peters (Division of Anti-Infective Drug Products reviewer and consultant to DSPIDP) that the pathologist selected to conduct the peer review was acceptable, but that "…FDA remains of the opinion that the studies need to be re-read to include at least all of the agreed upon…target tissues in addition to the [sic] all of the tumors, hyperplastic lesions, and tissues from at least 20% of the animals on the study (*cf. Facsimile Transmittal Sheet, DSPIDP, July 13, 2005*).
- On November 16, 2005, DSPTP informed the sponsor that:
    - "…all previously reported tumors and preneoplastic/hyperplastic lesions from all animals…and all the target tissues in all the animals…" should be reviewed.
    - At least 20% (not 10%, as proposed by the sponsor) "…of all tissues of all animals from both studies should be reviewed." [The basis for accepting review of 20% of all tissues, rather than all tissues as originally requested, is unclear.]
    - In addition to the list of target tissues (for non-neoplastic findings) proposed by the sponsor for re-examination, ocular tissue and liver sections should be examined for the mouse study.

Amended study reports for the 2-year mouse and rat carcinogenicity studies were submitted in the NDA. According to Dr. Siarey, the sponsor conducted the external peer review of tissues consistent with the approached agreed to by the Agency. For NDA 22-527, theses studies were reviewed by Drs. Siarey and Matthew Jackson (*cf. Statistical Review and Evaluation CARCINOGENICITY STUDIES NDA 21-527, Matthew Jackson, Ph.D., 6/25/2010*).

Mouse: in the 2-year carcinogenicity study in mouse (60/sex/group), FTY720 was administered orally by gavage at doses of 0, 0, 0.025, 0.25, and 2.5 mg/kg/day. Due to excessive mortality, dosing was discontinued in HDM during Week 92; mortality was slightly (not significantly) increased in HDF. Based on the Agency's independent analysis (*cf. Jackson, 6/25/2010*), the only statistically and/or biologically significant neoplastic finding was an increase in malignant lymphomas in MD and HD males and females (compared to pooled control data); group incidences are summarized in the following table (doses in mg/kg/day):

EVALUATION	**MALES**					**FEMALES**				
	0	**0**	**0.025**	**0.25**	**2.5**	**0**	**0**	**0.025**	**0.25**	**2.5**
original (2004)	4/60	8/60	5/60	11/60	18/60	15/60	21/60	17/60	26/60	26/60
peer review (2007)	1/60	8/60	5/60	10/60	18/60	10/60	14/60	12/60	25/60	24/60

Immunohistochemical staining of lymphocytes from C and HD animals (9/sex/group) with malignant lymphomas indicated no clear difference in the incidence of malignant B-cell or T-cell lymphomas.

While the external peer review reconciled the diagnostic inconsistencies, the high incidence of autolysis reported in the original study report, particularly in females, was not addressed. In females, "autolysis" was reported as a "general observation" for 13/60

CF, 5/60 CF, 12/60 LDF, 9/60 MDF, and 14/60 HDF or 22, 20, 15, and 23% of CF, LDF, MDF, and HDF, respectively. Overall, autolysis was reported for 53/300 or ≈18% of females. Exactly what this means is unclear. Unlike for males, "autolysis" or "partial autolysis" is not listed in the summary incidence tables for non-neoplastic lesions in females. On the contrary, for the majority of tissues, the number of females examined was ≥54/group. Tissues for which the fewest number of females examined per group were: eyes (45-54/group), gall bladder (43-52/group), parathyroid (44-56/group), thymus (45-54/group), Zymbal gland (22-47/group), and body cavity/bone/ear/teeth (total of <5 females for each organ/tissue).

In males, a "general observation" of "autolysis" was reported in a single HDM; however, the incidence of "partial autolysis" was notable. The table below summarizes the incidence of autolysis for those tissues/organs in which "partial autolysis" was reported in ≥10% of animals in any group. The majority of tissues affected were in animals that died prematurely, which might be expected. In fact, no organ/tissue was similarly affected in controls.

TISSUE	DOSE (mg/kg)				
	0	0	0.025	0.25	2.5
TOTAL					
general	0/60	0/60	0/60	0/60	1/60
salivary gland	0/60	6/60	6/60	7/60	1/60
stomach	3/59	10/60	10/59	11/60	6/59
duodenum	1/56	8/55	7/51	9/54	2/56
jejunum	3/60	8/57	9/55	7/53	2/56
ileum	2/55	6/56	8/54	9/51	2/51
colon	3/57	7/56	2/53	6/52	3/57
rectum	5/59	6/53	10/58	2/57	9/58
spleen	1/59	2/60	6/60	6/60	2/60
kidneys	7/60	5/60	9/60	8/60	5/60
urinary bladder	1/57	7/59	7/59	5/60	3/59
liver	4/60	5/60	7/60	5/60	4/60
seminal vesicles	0/60	5/60	8/60	7/60	3/60
brain	5/60	8/60	8/59	8/60	5/60
spinal cord	2/60	7/59	9/60	7/60	5/60
eyes	3/60	7/59	9/60	7/60	4/59

According to several sources (OECD, EPA, CFSAN Redbook 2000), an incidence of autolysis >10% in any one group or in a study compromises the validity of the study. Upon review of the study, the ExeCAC (*cf. Minutes dated 5/20/2010)* recommended that the Division ask the sponsor to "…address the high incidence of autolysis and the frequent miscoding of data in the electronic dataset, and…consider any other appropriate actions." However, according to the final statistical report (*Jackson, 6/25/2010)*,

> "…the high reported rates (98% or higher) of certain unexamined organs mean that meaningful analysis of carcinogenicity is not possible in the following cases:

For female mice: Body cavity, Bone, Mesenterium, Teeth
For male mice: Bone

> Among both male and female mice, there was a high incidence of autolysis. Noticably [sic] this was concentrated in a few organs. Among the female mice, the only organ which was found to be severely autolyzed (to the extent that a histopathological analysis was not possible) in more than 5% of the animals was the Duodenum (autolyzed in 20 animals). Among the male mice, the Cecum (34 animals), Duodenum (23 animals), Jejunum (21 animals) and Colon (15 animals) were all reported as being autolytic in at least 5% of animals."

According to this evaluation, the extent of autolysis would not appear to have seriously compromised the adequacy of either the male or female study. This is not consistent with the original concerns expressed about these studies, as previously described. Dr. Jackson also states that there were "…discrepancies in reporting missing and autolytic organs…", but it is unclear how extensive the problem was.

There is some question as to the exact meaning of the terms "partial autolysis" and "general observation" of "autolysis". In the original studies, three different pathologists conducted the original microscopic examinations. (b) (4) . The original Peer Review Pathologist, (b) (4), was replaced, at some point, by (b) (4). The Peer Reviewer(s) "…reviewed the draft report and a proportion of histological slides…" (The histopathology data provided in the final original report, dated 2004, was stated to reflect the "…mutually agreed-on diagnoses".) In addition, during the FDA-requested peer review process, an external pathologist (b) (4) reviewed slides from all tumors, all preneoplastic findings (focal hyperplasias), all slides from "…thymus, spleen, axillary and mesenteric lymph nodes, lungs, kidneys, bone marrow, eyes and liver…and all organs/tissues from 120 animals". In neither the original study report (2004) nor the amended final study report (March 29, 2007) did the sponsor provide a discussion of the high incidences of autolysis or partial autolysis and their impact on study validity and results. It is difficult to reconcile the high incidences of autolysis/partial autolysis with the fairly complete dataset provided for the study. The adequacy of the mouse study is important not only for assessment of the carcinogenic potential of FTY720, but also for that of metabolite M3 since it is likely to be the only carcinogenicity study that tests sufficiently high plasma levels of metabolite M3 (see Metabolite section of memo).

Overall, it would seem that the most likely explanation of the apparent discrepancy between the extent of the neoplastic and non-neoplastic findings and the reported incidence of autolysis or partial autolysis is strict reporting of autolysis by the original study pathologists. It is my understanding that the presence of autolysis does not necessarily preclude evaluation of tissues. Therefore, it is likely that, while autolysis or partial autolysis (however those terms were defined) may have been present, the pathologists were able to adequately examine affected tissues for neoplastic findings. One would assume that the high incidence of autolysis may have adversely affected the

evaluation of non-neoplastic findings, but since the primary purpose of a carcinogenicity study is to assess tumor formation, the mouse carcinogenicity study is acceptable. There was a clear signal for malignant lymphoma in both males and females. It would have been very helpful if the sponsor had directly addressed this issue.

Rat: in the 2-year carcinogenicity study in rat (51/sex/group), FTY720 was administered orally by gavage at doses of 0, 0, 0.05, 0.15, 0.5, and 2.5 mg/kg/day. Increased mortality was observed in HD animals, leading to cessation of dosing at Week 99 in males and Week72 in females. The HDF group was terminated early (Week 95), but HDM survivors were continued to scheduled sacrifice. Based on the reviews conducted by Drs. Siarey and Jackson, the ExeCAC concluded that the carcinogenicity study in rat was adequately conducted and negative for neoplastic lesions. The highest dose tested is ≈50 times the only dose recommended for humans (0.5 mg qd) on a mg/m^2 basis, and is associated with a plasma AUC 40-50 times that in humans at the RHD.

Genetic Toxicology: the genotoxic potential of FTY720 was tested in *in vitro* (Ames, chromosomal aberration in V79 [2 studies], mouse lymphoma tk) and *in vivo* (micronucleus in CD-1 mouse [i.p.] and Han:Wistar rat [p.o.]) studies. FTY720 was negative in the *in vitro* Ames, the *in vitro* mouse lymphoma tk, and *in vivo* micronucleus assays in mouse and rat. The *in vitro* chromosomal aberration assays were negative, but inadequate due to the lack of scoring of cells at a sufficient number of concentrations or at sufficiently cytotoxic concentrations. The *in vitro* mouse lymphoma tk assay was strictly a mutagenicity assay, since sensitivity to detect clastogenic effects was not confirmed (i.e., colony sizing was not performed).

The *in vivo* micronucleus assay in male mouse was conducted using i.p. dosing (6.25, 12.5, and 25 mg/kg); justification was not provided for the use of this route for a drug to be administered orally in humans. However, bone marrow exposure was demonstrated, with cytotoxicity (22% decrease in % PCE) evident at the HD at 24 hrs post dose and at all dose at 48 hrs (27, 11, 17% decrease in % PCE at LD, MD, and HD, respectively). The clinical route was used in the *in vivo* micronucleus assay in rat (20, 63, and 200 mg/kg), and marked bone marrow cytotoxicity was detected at all doses (40, 42, and 49% decrease in % PCE at LD, MD, and HD, respectively; and in the repeat experiment at 200 mg/kg [52, 65, and 80% decrease in PCE at 48, 72, and 96 hrs, respectively]).

Overall, the genetic toxicology battery is deficient in that clastogenic potential in *in vitro* assays in mammalian cells was not adequately examined, and additional *in vivo* studies to detect clastogenicity (i.e, other than the *in vivo* micronucleus assay) were not conducted. In addition, Dr. Siarey notes an additional deficiency in the *in vitro* assays, i.e., *in vitro* data indicated that little or no FTY720-P was formed by rat hepatocytes, suggesting inadequate assessment of indirect genotoxic effects. However, Dr. Siarey does not recommend that the sponsor conduct additional genotoxicity assays. I agree that no additional genotoxicity studies are needed since the carcinogenic potential has been adequately assessed in two species, and that the fingolimod-related tumor finding (i.e., malignant lymphoma) is consistent with a drug-related immunosuppressive effect.

Impurities: as Dr. Siarey notes in his review, the CMC review team identified a number of materials in the synthetic process or degradants of potential concern:

- Starting material: (b) (4)
- Synthetic intermediate: (b) (4)
- Impurities resulting from by-products of starting materials or synthetic process: (b) (4)
- Degradants: (b) (4)

For a drug substance and product administered at a total daily dose of 0.5 mg/day (the recommended clinical dose of fingolimod), the qualification threshold for drug substance impurities is (b) (4) and for drug product impurities is (b) (4)

The specification limit for (b) (4) is NMT (b) (4); while this is above the qualification threshold, it is acceptable since at the clinical dose (0.5 mg), a limit of NMT (b) (4) would result in a daily dose of (b) (4), which is acceptable even for a genotoxic impurity. The specification limit (NMT (b) (4)) for (b) (4) is also acceptable, for the same reason. The specification limit for (b) (4) was lowered to NMT (b) (4)

The sponsor agreed to lower the specification limit (originally NMT (b) (4)) for (b) (4) to NMT (b) (4) consistent with an acceptable daily intake. The specification limit for (b) (4) remains at NMT (b) (4). According to the CMC reviewer, this higher limit is qualified by use of drug batches Tox1/FTY and 00/1 in nonclinical studies, and taking into consideration the levels present in natural sources (b) (4) for the manufacture of related starting material. The nonclinical studies conducted to qualify impurities (including (b) (4) were inadequate; however, since the specification limit of NMT (b) (4) is consistent with those specified for natural sources, there would seem to be no need for additional nonclinical studies for these impurities.

Regarding the three potential degradants, the original specification (shelf-life) limit (NMT (b) (4) for (b) (4) was acceptable; however, that for (b) (4) (NMT (b) (4)) exceeded the qualification threshold. The sponsor has agreed to lower the limit to NMT (b) (4) (cf. *NDA 22-527 Quality Review #1 Addendum #2, Wendy I. Wilson-Lee, Ph.D., 9/1/2010*). Therefore, there is no safety concern for this degradant.

The sponsor conducted a number of *in vitro* and *in vivo* nonclinical studies to qualify identified process impurities and degradants. While Dr. Siarey has concluded that these studies do not fully qualify the impurities or degradants, no additional nonclinical studies are needed based on the lower specification limits set for each impurity or degradant identified as having a potential safety concern.

- **Pediatric Use**

The medical team is requiring a 24-month clinical trial in pediatric patients (10-17 years) as a PMR. To support the safety of this trial (and possible approval for use in the pediatric population), the sponsor will need to conduct a juvenile animal toxicology study in one species (rat). The sponsor submitted a juvenile study in rat to the NDA; however, that study was, by design, not an adequate study. It focused primarily on the immune system; no assessment of reproductive function or neurobehavioral development was conducted. Therefore, a juvenile animal study in rat should be conducted post-approval (as a PMR) to assess developmental effects as well as the standard toxicology parameters. The sponsor should submit a study protocol for feedback prior to study initiation.

- **Conclusions and Recommendations**

From a pharmacology/toxicology standpoint, the nonclinical studies provided for FTY720 are adequate to support approval, with appropriate labeling and a PMR for a juvenile animal toxicology study in one species (rat).

Recommended labeling:

2 page(s) of Draft Labeling have been Withheld in Full immediately following this page as B4 (CCI/TS)

Mouse (no severity scores in summary tables; doses in mg/kg/day)

STUDY	FINDING	MALES							
		0	0	0.025	0.1	0.25	0.5	2.5	5.0
13-wk	granulomatous reaction		0/9		0/10		0/10		5/10
	alveolar foam cells		0/9		0/10		0/10		1/10
"2-yr"	fibrosis	0/60	0/60	0/60		1/60		3/60	
	hyperplasia, bronchio-alveolar	1/60	5/60	3/60		0/60		1/60	
	alveolar macrophages	1/60	2/60	4/60		11/60		2060	
	osseous metaplasia	1/60	4/60	0/60		6/60		11/60	
	amyloidosis	9/60	14/60	9/60		17/60		16/60	
	inflammation	5/60	4/60	1/60		6/60		1/60	

STUDY	FINDING	FEMALES							
		0	0	0.025	0.1	0.25	0.5	2.5	5.0
13-wk	granulomatous reaction		0/10		0/10		0/10		4/10
	alveolar foam cells		0/10		0/10		0/10		0/10
"2-yr"	fibrosis	2/60	2/60	2/60		3/60		5/60	
	hyperplasia, bronchio-alveolar	2/60	3/60	3/60		8/60		5/60	
	alveolar macrophages	7/60	8/60	10/60		16/60		30/60	
	osseous metaplasia	0/60	1/60	0/60		1/60		5/60	
	amyloidosis	8/60	3/60	5/60		16/60		18/60	
	inflammation	4/60	4/60	6/60		7/60		11/60	

Rat (doses in mg/kg/day)

STUDY	FINDING	MALES														
		0	0.01	0.03	0.05	0.1	0.15	0.3	0.5	1.5	2.5	5	7.5	10	30	60
1-month	none (0.003-3 mg/kg)															
1-month	edema (slight)	0/10												0/10	0/10	2/4
	alveolar macrophages															
	slight	0/10												0/10	9/10	3/4
	moderate	0/10												0/10	1/10	1/4
13-wk	smooth muscle hypertrophy															
	grade 1	0/10				0/10			0/10			6/10				
	grade 2	0/10				0/10			0/10			4/10				
	collagenization, inter															
	grade 1	0/10				3/10			8/10			10/10				
	alveolar macrophages															
	grade 1	2/10				6/10			8/10			7/10				
	grade 2	0/10				0/10			0/10			3/10				
	infiltrates, granulocytes															
	grade 1	3/10				2/10			5/10			4/10				
	grade 2	0/10				1/10			0/10			3/10				
26-wk	cell infiltration, macrophage (slight)	0/11	0/11	0/12				0/12						1/10		
	smooth muscle hypertrophy (slight)	0/11	0/11	0/12				0/12						7/10		
26-wk	smooth muscle hypertrophy															
	main study	0/20						2/20		10/20			19/20			
	recovery	0/10						--		3/10			4/9			
	osseous metaplasia															
	main study	0/20						1/20		1/20			2/20			
	recovery	0/10						--		0/10			1/9			
"2-yr"	smooth muscle hypertrophy	1/102			0/51		0/51		11/51		38/51					
	(average grade)	(1.0)			--		--		(1.3)		(1.2)					
	pleural thickening	1/102			1/51		9/51		26/51		32/51					
	(average grade)	(1.0)			(1.0)		(1.0)		(1.1)		(1.4)					
	osseous metaplasia	3/102			2/51		1/51		6/51		8/51					
	(average grade)	(1.0)			(1.0)		(1.0)		(1.2)		(1.4)					

STUDY	FINDING	FEMALES														
		0	0.01	0.03	0.05	0.1	0.15	0.3	0.5	1.5	2.5	5	7.5	10	30	60
1-month	none (0.003-3 mg/kg)															
1-month	edema (slight)	0/10												0/10	1/10	0/2
	alveolar macrophages															
	slight	0/10												0/10	710	2/2
	moderate	0/10												0/10	1/10	0/2
13-wk	smooth muscle hypertrophy															
	grade 1	0/10				0/10			0/10			6/10				
	grade 2	0/10				0/10			0/10			4/10				
	collagenization, inter															
	grade 1	0/10				8/10			9/10			10/10				
	alveolar macrophages															
	grade 1	1/10				3/10			3/10			5/10				
	grade 2	0/10				0/10			0/10			5/10				
	infiltrates, granulocytes															
	grade 1	1/10				3/10			4/10			3/10				
	grade 2	0/10				1/10			0/10			7/10				
26-wk	cell infiltration, macrophage (slight)	0/12	0/12	0/12				0/12						0/12		
	smooth muscle hypertrophy (slight)	0/12	0/12	0/12				0/12						9/12		
26-wk	smooth muscle hypertrophy															
	main	0/20						3/20		16/21			20/20			
	recovery	0/10						--		1/9			4/8			
	osseous metaplasia															
	main	0/20						0/20		0/20			0/20			
	recovery	0/10						--		0/9			0/8			
"2-yr"	smooth muscle hypertrophy	0/102			2/51		5/51		6/51		22/51					
	(average grade)	--			(1.5)		(1.6)		(1.3)		(1.1)					
	pleural thickening	3/102			0/51		2/51		5/51		8/51					
	(average grade)	(1.0)			--		(1/0)		(1.0)		(1.3)					
	osseous metaplasia	0/102			0/51		0/51		0/51		0/51					

Dog (doses in mg/kg/day)

STUDY	FINDING	MALES							
		0	0.001	0.01	0.1	1	3	10	30
1-mo	focal bronchiolar alteration, slight	0/5	0/3	0/5	1/5	0/3			
	edema								
	very slight	0/5	0/3	0/5	0/5	1/3			
	slight	0/5	0/3	0/5	0/5	1/3			
	moderate	0/5	0/3	0/5	0/5	1/3			
	macrophage accumulation								
	very slight	0/5	0/3	0/5	2/5	0/3			
	slight	0/5	0/3	0/5	1/5	0/3			
	moderate	0/5	0/3	0/5	0/5	3/3			
	subpleural fibrosis, slight	0/5	0/3	0/5	1/5	0/3			
	inflammatory cell infiltration, very slight	0/5	0/3	0/5	0/5	3/3			
	pneumonia								
	slight	0/5	0/3	0/5	0/5	1/3			
	moderate	0/5	0/3	0/5	0/5	2/3			
	hemorrhage, very slight	0/5	0/3	0/5	0/5	2/3			
1-mo	focal bronchiolar alteration								
	very slight	0/5					0/3	0/5	1/5
	slight	0/5					0/3	2/5	0/5
	edema								
	very slight	0/5					0/3	1/5	0/5
	slight	0/5					2/3	2/5	0/5
	moderate	0/5					1/3	0/5	0/5
	cell infiltration, macrophage								
	very slight	0/5					0/3	0/5	2/5
	slight	0/5					0/3	1/5	1/5
	moderate	0/5					3/3	2/5	0/5
	cell infiltration, neutrophil								
	very slight	0/5					1/3	0/5	0/5
	slight	0/5					2/3	1/5	0/5
	deposit of fibrin								
	very slight	0/5					0/3	1/5	0/5
	slight	0/5					2/3	2/5	0/5
	moderate	0/5					1/3	0/5	0/5
	subpleural fibrosis								
	slight	0/5					0/3	1/5	0/5
	moderate	0/5					0/3	1/5	0/5
	hyaline membrane, slight	0/5					1/3	1/5	0/5
	pneumonia								
	very slight	0/5					0/3	1/5	1/5
	slight	0/5					2/3	0/5	0/5
	moderate	0/5					1/3	1/5	0/5
6-mo	congestion, slight	0/4	0/4	0/4		0/4		3/4	
	edema, slight	0/4	0/4	0/4		1/4		0/4	
	cell infiltration, macrophage								
	very slight	0/4	1/4	0/4		0/4		1/4	
	slight	0/4	0/4	0/4		2/4		1/4	
	cell infiltration, neutrophil, moderate	0/4	0/4	0/4		1/4		0/4	
	pneumonia, moderate	0/4	0/4	0/4		1/4		0/4	

STUDY	FINDING	FEMALES							
		0	0.001	0.01	0.1	1	3	10	30
1-mo	focal bronchiolar alteration								
	very slight	0/5	0/3	1/5	0/5	0/3			
	moderate	0/5	0/3	1/5	0/5	0/3			
	edema								
	very slight	0/5	1/3	0/5	0/5	1/3			
	slight	0/5	0/3	0/5	0/5	1/3			
	macrophage accumulation								
	very slight	0/5	0/3	0/5	1/5	0/3			
	slight	0/5	0/3	0/5	1/5	0/3			
	moderate	0/5	0/3	0/5	0/5	3/3			
	subpleural fibrosis, slight	0/5	0/3	1/5	0/5	0/3			
	inflammatory cell infiltration								
	very slight	0/5	1/3	0/5	1/5	0/3			
	slight	0/5	0/3	0/5	0/5	1/3			
	pneumonia								
	slight	0/5	0/3	0/5	1/5	2/3			
	moderate	0/5	1/3	0/5	0/5	1/3			
	hemorrhage								
	very slight	0/5	1/3	0/5	0/5	1/3			
	slight	0/5	0/3	0/5	1/5	0/3			
1-mo	focal bronchiolar alteration, slight	0/5					0/3	0/5	1/5
	edema								
	very slight	0/5					0/3	1/5	0/5
	slight	0/5					3/3	1/5	0/5
	cell infiltration, macrophage								
	very slight	0/5					0/3	0/5	1/5
	slight	0/5					1/3	2/5	1/5
	moderate	0/5					2/3	2/5	0/5
	cell infiltration, neutrophil, slight	0/5					0/3	1/5	0/5
	deposit of fibrin								
	very slight	0/5					0/3	1/5	0/5
	slight	0/5					3/3	1/5	0/5
	subpleural fibrosis, slight	0/5					0/3	0/5	1/5
	hyaline membrane, slight	0/5					0/3	1/5	0/5
	pneumonia								
	very slight	0/5					1/3	0/5	1/5
	slight	0/5					2/3	2/5	0/5
6-mo	edema								
	very slight	0/4	0/4	0/4		0/4		1/4	
	slight	0/4	0/4	0/4		1/4		0/4	
	cell infiltration, macrophage								
	very slight	0/4	0/4	0/4		1/4		0/4	
	slight	0/4	0/4	0/4		1/4		2/4	
	moderate	0/4	0/4	0/4		0/4		1/4	
	pneumonia, slight	0/4	0/4	0/4		1/4		0/4	

Monkey (doses in mg/kg/day)

STUDY	FINDING	MALES					
		0	0.5	1	3	10	30
13-wk	smooth muscle hyperplasia, focal, slight	0/2			0/2	0/2	1/2
52-wk	pneumonitis						
	minimal	4/4		3/4	3/4	2/4	
	slight	0/4		1/4	1/4	2/4	
	prominent alveolar macrophages, minimal	0/4		0/4	0/4	2/4	
	aggregation of alveolar macrophages						
	minimal	0/4		0/4	0/4	1/4	
	slight	0/4		0/4	0/4	0/4	
	smooth muscle hypertrophy at bronchiolar-alveolar junction						
	minimal	0/4		1/4	0/4	0/4	
	slight	0/4		3/4	3/4	1/4	
	moderate	0/4		0/4	1/4	3/4	
	alveolar hyperdistension						
	minimal	0/4		0/4	3/4	0/4	
	slight	0/4		0/4	0/4	2/4	
	moderate	0/4		0/4	0/4	2/4	
	extension of bronchiolar epithelium into respiratory bronchioles, minimal	0/4		0/4	1/4	1/4	
	apparent alveolar collapse with pneumonitis	0/4		0/4	0/4	1/4	
39-wk	distended alveolus, focal	1/4	3/4		4/4		
	smooth muscle hypertrophy						
	minimal	0/4	2/4		2/4		
	slight	0/4	0/4		2/4		
	increased collagen						
	minimal	1/4	4/4		1/4		
	slight	0/4	0/4		2/4		

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/s/

LOIS M FREED
09/20/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 22-527
Supporting document/s:	SDN 1, SDN 6
Applicant's letter date:	21 December 2009
CDER stamp date:	21 December 2009
Product:	Gilenya® (Fingolimod HCl)
Indication:	Multiple Sclerosis
Applicant:	Novartis Pharmaceuticals Corp
Review Division:	Division of Neurology Products
Reviewer:	Richard Siarey
Supervisor/Team Leader:	Lois Freed
Division Director:	Russell Katz
Project Manager:	Hamet Toure

Disclaimer

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TABLE OF CONTENTS

1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Lung toxicity of fingolimod was observed in animal species, with NOAEL's less than the recommended human dose for rat (0.2 mg/m^2) and dog (0.18 mg/m^2) that, in the rat, were reduced with increase in study duration; no NOAEL was established for monkey. The lesions included smooth muscle hypertrophy in small terminal bronchioles, alveolar ducts and the entrance to alveolar sacs, increased collagen, increased alveolar macrophages and lungs that did not collapse at necropsy. These lesions appear to change lung function as dyspnea and labored breathing were observed. In the 52 week monkey study, extreme breathing difficulties were observed in several monkeys both after treatment and while under ketamine (an anesthetic that suppresses breathing much less than most others) sedation and resulted in 1 animal developing a hypoxia/blue appearance, requiring placement into an incubator for oxygenation. In the rat, airway obstruction was also observed acutely, and was potentiated by ketamine. Therefore, although partial reversibility of lung toxicity was observed, lung toxicity was observed in all nonclinical species, suggesting that chronic administration of fingolimod could cause functional changes and it is recommended, from the nonclinical perspective, that fingolimod not be approved.

1.1.2 Additional Non Clinical Recommendations

There are notable deficiencies in the mouse carcinogenicity and reproduction and developmental toxicity sections, where there are several invalid studies. There is a lack of coverage for metabolites in patients with impaired function of certain organs, such as kidneys, where there are increased levels of metabolites.

1.1.3 Labeling

1.2 Brief Discussion of Nonclinical Findings

Fingolimod is a structural analog of sphingosine, an 18-carbon amino alcohol that forms a primary part of sphingolipids, a class of cell membrane lipids. Similar to sphingosine, fingolimod is phosphorylated by sphingosine kinases to the active moiety fingolimod-phosphate. Fingolimod has high affinity at G protein-coupled sphingosine-1-phosphate (S1P) receptors; $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$ and acts at these receptors depriving lymphocytes of the obligatory signal to egress from lymphoid organs and, thus, prevents the recirculation of these cells to other sites, sequestering them in secondary lymphoid tissue, to prevent recirculation of lymphocytes to other sites. In animal models of multiple sclerosis, fingolimod has been demonstrated to delay the onset of acute clinical signs and prevent relapses.

In animals, the primary target organs associated with fingolimod are the lungs and heart. In rodent, dogs and non-human primates, lung weight was increased, and histopathological findings included smooth muscle hypertrophy, increased collagen and occasional observations of fibrin that may explain the gross finding of increased incidences of lungs not collapsing at necropsy in toxicity studies of all species tested. Other histopathological findings included osseous metaplasia and plural thickening. These changes were generally observed in small terminal bronchioles, alveolar ducts and the entrance to alveolar sacs, areas important for lung function. These structural changes may explain the incidences of labored breathing, dyspnea and rales in all species, and the extreme breathing difficulties observed in several monkeys while under sedation that resulted in 1 animal developing a hypoxia/blue appearance and requiring placement into an incubator for oxygenation. NOAELs for lung toxicity were 1.5, 0.18, 0.2 and <6 mg/m^2, for mouse, rat, dog and monkey, respectively making the rat and dog the most sensitive species for lung toxicity, although the relative sensitivity of monkey is uncertain due to the lack of a NOAEL. The human dose on a body surface area basis is 0.31 mg/m^2.

Fingolimod's actions on the heart included an acute increase in blood pressure and decrease in heart rate. In addition, affects on the papillary muscle (wall thickening) was observed in dogs at relatively high doses. In the reproductive and development studies, fetuses were not assessed at all doses. The studies do, however, highlight the fact that fingolimod is teratogenic in rats and lethal to fetuses in both rats and rabbits, without a clear NOAEL for fetal toxicity in rats. Fingolimod crosses the placenta and has been found in milk of lactating animals.

During the review process, the CMC reviewer identified 10 potentially genotoxic impurities and degradants. Adequate studies to evaluate the genotoxic potential of these impurities and degradants have not been submitted, however if the specification limit is not exceeded, (b) (4), this should not be of concern. Metabolites, M2 and M3, were identified as the primary circulating metabolites in humans, but neither accounted for ≥10% of total drug-related material in plasma in otherwise healthy patients. However, increases in plasma levels of these metabolites were found to occur in certain patient populations (e.g., renal or hepatic impairment), for whom adequate exposure data have not been submitted.

2 Drug Information

2.1 Drug:

Gilenya®.

2.1.1 CAS Registry Number: 162359-56-0

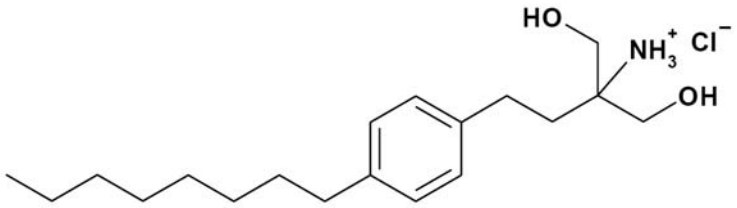
2.1.2 Generic Name: Fingolimod

2.1.3 Code Name: FTY720

2.1.4 Chemical Name: 2-Amino-2-(2-(4-octylphenyl)ethyl)propan-1,3-diol hydrochloride

2.1.5 Molecular Formula/Molecular Weight: $C_{19}H_{33}NO_2 \cdot HCl$; 343.93 (hydrochloride salt form), 307.48 (salt-free form), Salt/base ratio: 1.119.

2.1.6 Structure:



2.1.7 Pharmacologic class: Sphingosine-1-phosphate receptor modulator.

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 57,293 (DAIDP, Prophylaxis of organ rejection; withdrawn 19 Feb 2008).
IND 70,407 (DAVP, Treatment of hepatitis C; withdrawn 11 Jul 2005).
IND 70,139 (DNP, Treatment of multiple sclerosis; submitted for NDA 22-527 21 Dec 2009).

2.3 Clinical Formulation:

Hard capsule containing white to almost white powder.

2.3.1 Drug Formulation: Hard gelatin capsule.

Comments on Novel Excipients
Excipients of the drug product formulation include mannitol and magnesium stearate (vegetable origin). Each capsule contains the following excipients: gelatin, titanium dioxide and iron oxide (yellow). These excipients comply with compendial or regulatory standards (see CMC review, 30 June 2010).

Comments on Impurities/Degradants of Concern
The CMC reviewer (30 June 2010) noted that the proposed shelf-life limit (NMT (b) (4) for the drug product degradan (b) (4) exceeds the ICH qualification threshold (NMT 1.0%). (b) (4) is found in the drug product as a result of an interaction of the (b) (4). As the drug

substance, not the drug product was used in nonclinical studies there are no data on (b) (4)

2.4 Proposed Clinical Population and Dosing Regimen

The proposed clinical population for fingolimod is individuals with relapsing forms of multiple sclerosis; the dosing regimen is 0.5 mg once daily administered orally.

2.5 Regulatory Background

Fingolimod was first submitted to the Agency (DAIDP; 19 Nov 1998) as an IND for renal transplantation (IND 57,293). Under that IND, fingolimod was evaluated in renal transplantation patients at doses of 2.5 mg and 5 mg/day, in combination with cyclosporine and steroids. The renal transplantation clinical program included two phase 3 trials, but was discontinued (5 Aug 2008), as fingolimod combined with cyclosporine A did not show increased efficacy compared to mycophenolate mofetil combined with cyclosporine A (standard-of-care). Another IND (IND 70,407) was submitted (DAVP; 13 Aug 2004) for the treatment of hepatitis C, but was withdrawn (22 Jul 2005).

IND 70,139 for fingolimod was submitted on 21 Oct 2004 for treatment of multiple sclerosis. Fingolimod was submitted as a rolling NDA on 16 June 2009, with a final submission date of 21 December 2009. Fingolimod was given fast track status. The original action date (21 June 2010) was extended by 3 months due to submission of a major amendment. An advisory committee met to consider fingolimod for treatment of MS on 10 June 2010.

3 Studies

3.1 Studies

Pharmacodynamics Studies

- To demonstrate that FTY720 phosphate is the active moiety.
- Several studies of the effects on the acute and chronic relapsing experimental autoimmune encephalomyelitis (EAE) in rats (Wistar and Lewis) and mice (SJL/J).
- *In vitro* safety pharmacology profile.

Safety Pharmacology Studies

- Several neurological studies for CNS function.
- Several studies assessing cardiovascular function.
- Studies to assess respiratory system.
- Others studies on platelet aggregation, GI tract and renal function.

ADME

- Absorption, distribution, metabolism and excretion in mice, rats, dogs and monkey.

Pharmacokinetics, Drug interactions Studies

- Determination and toxicokinetics of FTY720 and RAD001, FTY720 and ERL080 and FTY720 and methotrexate in blood.

Single-dose toxicity Studies

- Acute single dose studies after intravenous and oral administration to mice, rats and dogs.

Repeat-dose toxicity Studies

- Repeat oral dose studies in mice (13-week), rats (1 month, 13 weeks and 26 weeks), dogs (1 and 6 months) and Cynomolgus monkeys (2, 13, 39 and 52 weeks).

Genotoxicity Studies

- Reverse mutation (AMES) test.
- Mutation assay at the thymidine kinase locus.
- Chromosomal aberration test.
- Mouse bone marrow micronucleus test.

Carcinogenicity Studies

- 104-Week oral (gavage) carcinogenicity study in mice and rats.

Reproductive and developmental toxicology Studies

- An oral combined fertility and early embryonic development study in rats.
- Oral embryo-fetal development study in rats.
- Oral embryo-fetal development dose-range finding study in rabbits.
- An oral pre- and postnatal development study in rats.

Other Toxicity Studies

- Antigenicity
- Immunotoxicity
    - Assessment of immunosuppressive potential during contact allergy.
    - Assessment of antibody response to SRBC.
- Mechanistic
    - Investigation of the effects on rat versus mouse endothelial cell lines.
    - Investigations of the influence of FTY720 on the cytokine production by lung epithelial cells and alveolar macrophages of the rat.
    - Effect on the vascular tone of rat thoracic aortic rings.
    - Effects on cultured primary neonatal rat cardiomyocytes *in vitro*.
- Impurities
    - Ames test.
    - Chromosomal aberration test.
    - Micronucleus test *in vitro*.
    - 4-Week oral (gavage) impurity toxicity study in rats.

3.2 Studies Not Reviewed

Pharmacokinetics

- Analytical methods.

Single-dose toxicity Studies
Studies by the intravenous route.
Repeat-dose toxicity Studies

- Preliminary dog study.

Reproductive and Developmental toxicology Studies

- A subchronic oral (gavage) toxicity study of FYY720 in neonatal and juvenile albino rats.

Other Toxicity Studies

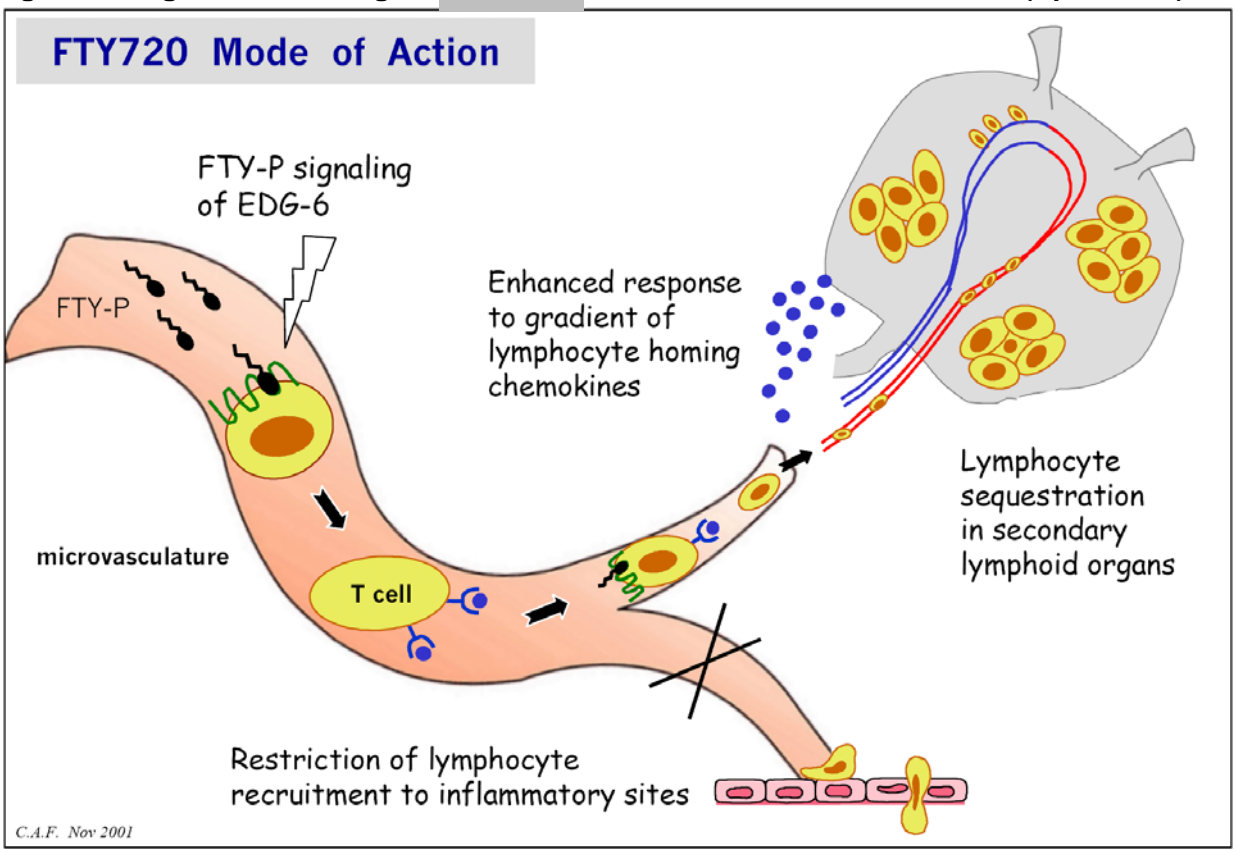
4 Pharmacology

4.1 Primary Pharmacology

FTY720 is a structural analog of sphingosine, an 18-carbon amino alcohol that forms a primary part of sphingolipids, a class of cell membrane lipids. Similar to sphingosine, FTY720 is phosphorylated by sphingosine kinases to the active moiety FTY720-phosphate (FTY720-P). FTY720 is a racemate, with the (S)-enantiomer (AML629) being the more active enantiomer. An *in vitro* binding study, to detect agonist-induced GTP[γ-35S] binding, demonstrated that the (S)-configured FTY720-P enantiomer has high affinity for G protein-coupled sphingosine-1-phosphate (S1P) receptors; EC_{50} values for these receptors were $S1P_1$ (0.3 nM), $S1P_3$ (3.1 nM), $S1P_4$ (0.6 nM) and $S1P_5$ (0.3 nM); there was little affinity for $S1P_2$ (EC_{50} >10 µM).

FTY720 acts at S1P-receptors on thymocytes, pneumocytes and lymphocytes to induce abnormal internalization of the receptor. This action of FTY720 causes these cells to become insensitive to S1P, depriving the cells of the obligatory signal to egress from lymphoid organs and, thus, prevents the recirculation of these cells to other sites.

Figure 1 Diagram illustrating the (b) (4) ***in vivo* mechanism for FTY720 (Sponsor's)**



Effects of FTY720 on autoimmune encephalomyelitis (EAE) models

Daily treatment of F Wistar rats with FTY720 (0.3 mg/kg, oral) for 14 days completely prevented the onset of acute EAE clinical signs and oral doses of 0.1-0.9 mg/kg daily for 13 Days (Day 16-29) prevented relapses in F Lewis rats with established signs of EAE. These effects were due to the (S)-enantiomer; the (R)-enantiomer provided no protection. FTY720 given for only 7 days protected from signs of EAE until Day 5 (i.e. Day 12) post removal of treatment. Treatment of the acute phase of EAE in F Lewis rats with oral doses of 0.3 mg/kg FTY720 daily, every other day or every third day, prevented severe clinical signs of EAE, in contrast to the severe signs observed in weekly treated animals. The increase in vascularity of the lumbar area of the spinal cord and increased inflammation composed of T-cells and macrophages observed during relapse were reduced by FTY720.

In F SJL/J mice, oral administration of 0.6 mg/kg of FTY720 5 times a week prevented further relapse of clinical signs of EAE, in contrast to vehicle and β-interferon-treated animals.

4.2 Secondary Pharmacology

FTY720 was tested in a panel of *in vitro* radioligand binding assays for 66 GPCRs, transporters, ion channels and enzymes. FTY720 had affinity for a number of targets, with K_i values of 1-10 μM at human receptors: Adenosine Ad_3, adrenergic α_{2A}, α_{2B}, α_{2C}, β_1, cannabinnoid CB_1, cholecystokinin CCK_b, dopamine D_1, D_{2L}, D_3, D_5, histamine H_1, H_3, motilin, muscarinic M_5, melanocortin MC_3, MC_4, NT_1, NK_1, opiate κ, μ, serotonin $5HT_{1A}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, dopamine and noradrenaline transporters, PDE4d. The affinity for the histamine H_2 receptor was higher, with a K_i of 0.5 μM. FTY720-P showed no affinity at these targets up to 10 μM.

When investigated on guinea pig ileum and trachea, FTY720, FTY720-P and S1P did not trigger or modulate contractile responses to carbachol and histamine. This suggests that there was no effect on cholinergic or histaminic responses.

4.3 Safety Pharmacology

At single oral doses of up to 10 mg/kg, FTY720 had no effect on alertness, locomotor activity or other behaviors in mice; however, in the rotorod test, FTY720 significantly decreased performance at 3 and 10 mg/kg. In rats, FTY720 given up to 30 (4 days) plus 12 mg/kg/day (3 days) prolonged phenobarbital-induced sleep, but had no effect on locomotor activity and did not induce convulsions. In contrast, FTY720 (10 mg/kg) had no effect on hexobarbital-induced sleep in mice. FTY720-treated mice did not exhibit any pro- or anti-convulsant activities or affect diazepam-induced protection to metrazol-induced seizures. In rats, there was no change in nerve conduction; however, a reduction in twitch amplitude of the gastrocnemius muscle was observed in rabbits after a 10 mg/kg dose. It is unclear as to whether these effects were a direct action on the muscle, produced at the neuromuscular junction, or due to changes in nerve conduction.

The hERG tail current was inhibited to a similar extent by FTY720 (0.5 μM) and FTY720-P (0.5 μM) (25% and 18%, respectively); IC_{50}'s were not calculated. Action potentials evoked from sheep Purkinje fibers were not affected by FTY720 (≤ 100 ng/ml or 3 μM) alone or in combination with 100 ng/ml RAD N BHT, suggesting a lack of effect on QT interval. In pig hearts, QT interval was assessed *in vitro,* and neither FTY720 nor FTY720-P had an effect at concentrations up to 500 nM. In 2 experiments using rabbit sinus nodes, no effect on the action potential was observed after perfusion with 1 μM FTY720, but spontaneous beating frequency was reduced. However, these data are unreliable, as the presence of FTY720 was not detected in the bathing solution. Cerebral blood flow and autoregulation was unaffected by FTY720 (30 mg/kg) in rats.

FTY720 administered i.d. at doses up to 10 mg/kg had no effect on arterial blood pressure, heart rate or cardiac contractility in dogs; however, when FTY720 was administered at 1 mg/kg i.v. or orally, there was an increase in blood pressure. Monkeys given 1 and 10 mg/kg FTY720 had a decreased heart rate 8 hours post-dose and increased blood pressure from 4-24 hours post dose. In cats, FTY720 at doses up to 10 mg/kg had no affect on basal heart rate, blood pressure, contraction of the nictitating membrane or blood pressure, or on heart rate responses to carotid occlusion and adrenaline treatment.

Incubation of cardiac myocytes with 10 μM M3 produced no change in contractions or creatine kinase levels, suggesting a lack of cytotoxicity or apoptotic activity. In rats, M3 (10 mg/kg, i.v.) produced no change in blood pressure, heart rate, respiration or QTc, alone (n = 3) or in combination (n = 2) with metoprolol (100 mg/kg, oral). M30 (10 μM) had no agonistic activity at any S1P receptor subtypes, and did not induce an apoptotic response in human T-cells.

In monkey, respiratory rate, oxygen saturation, arterial oxygen and carbon dioxide tension were not changed following single oral doses up to 10 mg/kg. Renal changes observed in rats included a decrease in urine volume over the first 4 hours post-dose (1 mg/kg) and changes to renal function that suggested a decrease in glomerular filtration rate and an increase in sodium reabsorption. In rodent, there was no change in GI motility or gastric emptying after 4-day repeat 30 mg/kg followed by 3-day repeat 12 mg/kg FTY720 or on gastric electrolyte levels after 3 mg/kg FTY720. In addition, FTY720 at concentrations up to 1 μM produced no significant effect on agonist-induced or basal guinea-pig ileum contractions.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

The Sponsor's tables (provided below) summarize the results of the PK/ADME data. Oral bioavailability of FTY720 was high in all species; 66% in mice, 59% in rats, 61% in dogs, 51% in monkeys. Human bioavailability was estimated at 93%. Inter-conversion

of FTY720 with FTY720-P in animals and human does not allow an accurate estimate of oral bioavailability; however, blood C_{max}'s and disappearance of FTY720 and FTY720-P were approximately in parallel and the AUC ratio for human was 0.54. Absorption of FTY720 and FTY720-P in animals after oral administration was fairly extensive (50-91% in animals, 85% in humans), but slow, with T_{max} at 8-24 hours post-dose. FTY720 and FTY720-P were cleared approximately in parallel from the blood with $t_{½}$ values ranging from 14-44 and 21-78 hours for FTY720 and FTY720-P, respectively. Analysis of the enantiomers of FTY720-P in blood from mouse, rat, rabbit, dog and monkey demonstrated that the pharmacologically active (S)-enantiomer (AML629) was the most prominent (≥ 94%), with the (R)-enantiomer (AML627) being below the limit of detection (limit of detection 1-6% of total FTY720 in the sample). AML629 was also exclusively found in human blood. Similarly, only AML629 was detected in rat brain, with AML627 below the limit of detection. When [^{14}C]AML627 was administered to rats, AML627 was rapidly de-phosphorylated to FTY720 and re-phosphorylated to AML629, with only 10% of FTY720-P present as AML627 after 1 hour post-administration.

Table Pharmacokinetic parameters of FTY720 in blood after a single oral dose of FTY720 in various species (Sponsor's)

PK parameters [$]	Mouse	Mouse		Rat	Rat			Dog	Dog	Monkey				Monkey	Human
Sex	m	m	m	m	m	m	m	m	m	m	f	m	f	m	m/f
Dose (mg/kg)	2.5	0.1	1.0	7.5	0.1	1.0	3.0	1.0	3.0	0.1	0.1	1.0	1.0	3.0	0.0181 [a]
Radiolabel	^{14}C	-	-	^{14}C	-	-	-	^{14}C	^{14}C	-	-	-	-	^{14}C	-
T_{max} (h)	8	12	12	8	12	12	12	8	9.3	9.3	14.7	6.7	8.0	9.3	12
C_{max} (ng/mL)	95.3	6.90	57.5	148	3	20	70.8	30.7	165	1.5	1.7	16.4	18.2	30.7	1.1
C_{max} / dose (ng/mL)/(mg/kg)	38.1	69.0	57.5	19.7	30	20.4	23.6	30.7	55.0	15.0	17.0	16.4	18.2	10.2	60.9
AUC (ng·h/mL)	3167	275	2491	6795	63	627	1719	1390	8491	68	91	676	934	1640	201
AUC / dose (ng·h/mL)/(mg/kg)	1267	2750	2491	906	631	627	573	1390	2830	677	912	676	934	547	11127
AUC interval (h)	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf
Apparent terminal half-life $t_{1/2}$ (h)	25	31.7	34	30.4	13.6	25.1	15.6	26	29.3	30.2	33.8	30.9	33.3	44	146
Calculated accumulation factor (R_a)	2.06 [b]	2.45	2.58	2.37 [b]	1.4 [b]	2.1 [b]	1.54	2.12 [b]	2.31	2.36	2.58	2.4	2.55	3.2 [b]	9.3
Bioavailability (% of dose)	66	73.1	66.3	59	70.9	70.4	64.4	n.a.	60.8	35.1	45.6	n.a.	n.a.	51	93
Reference:	[R0100826]	[RR-K00505-1]		[R01-0622]	[R-7766]			[R0300770]	[R-770]	[R-7567]				[R01-623-03]	[A0108]

$: to obtain µmol units, divide ng units by the molecular weight of FTY720 (307.48).

n.a.: not assessed. 0-inf: time period of zero to infinity.

a: an oral dose of 1.25 mg was given to 5 male and 6 female healthy volunteers, mean body weight 69.2 ± 10.8 kg.

b: calculated by: $R_a = 1/(1-\exp(-\ln 2/t_{1/2}\cdot\tau))$, τ = dosing interval (=24 h).

Source: **mouse** [Table 2.6.5.3A-Study R0100826] [Table 2.6.5.3B-Study RR-K00505-1], **rat** [Table 2.6.5.3C-Study R01-0622] [Table 2.6.5.3E-Study R-7766]; **dog** [Table 2.6.5.3L-Study R0300770] [Table 2.6.5.3N-Study R-7703]; **monkey** [Table 2.6.5.3Q-Study R-7567] [Table 2.6.5.3R-Study R01-623-03]; **human** [Study A0108]

Table of Pharmacokinetic parameters of FTY720 in blood after a single intravenous dose of FTY720 in various species (Sponsor's)

PK parameters [$]	Mouse	Mouse	Rat	Rat	Dog	Monkey	Monkey	Monkey	Human
Sex	male	male	male	male	male	male	female	male	m/f
Dose (mg/kg)	2.5	1	4	1	1	0.1	0.1	0.1	0.0145 [a]
Radiolabel	^{14}C	-	^{14}C	-	-	-	-	^{14}C	-
AUC (ng·h/mL)	5442	3760	6027	890	4658	197	197	323	175
AUC / dose (ng·h/mL)/(mg/kg)	2177	3760	1507	890	4658	1973	1971	3230	12110
AUC interval	0-inf	0-inf	0-168 h	0-inf	0-inf	0-inf	0-inf	0-inf	0-inf
Apparent elimination half-life $t_{1/2}$ (h)	27.8	33.5	22.9	15.8	24.6	29.5	31.0	35	144.0
Accumulation factor (R_a)	2.22 [b]	2.55	1.94	1.54 [b]	2.04	2.32	2.41	2.64 [b]	9.17 [b]
MRT (h)	31.5 [c]	37.1 [c]	32	26.8 [c]	34.7 [c]	27.2 [c]	29.4 [c]	36.4 [c]	n.a.
CL (L/h/kg)	0.55	0.266	0.666	1.14	0.219	0.459	0.473	0.330	0.0917
CL (mL/min/kg)	9.17	4.43	11.1	19.0	3.65	7.65	7.89	5.50	1.528
Hepatic extraction ratio E_H (%) [d]	10%	5%	20%	34%	12%	18%	18%	13%	7%
V_{ss} (L/kg)	17.30	9.86	21	30.5	7.60	12.5	13.9	12.0	17.3
Study number	[R0100826]	[RR-K00505-1]	[R01-0622]	[R-7766]	[R-7703]	[R-7567]	[R-7567]	[R01-623-03]	[A0108]

$: to obtain µmol units, divide ng units by the molecular weight of FTY720 (307.48). n.a.: not assessed. 0-inf: time period of zero to infinity.

a: an oral dose of 1 mg was injected to 5 male and 6 female healthy volunteers, mean body weight 69.2 ± 10.8 kg.

b: calculated by: $R_a = 1/(1-\exp(-\ln 2/t_{1/2}\cdot\tau))$, τ = dosing interval (=24 h).

c: calculated by: MRT = V_{ss}/CL.

d: calculated by: E_H (%) = 100 · CL / Q_H, where Q_H= hepatic blood flow from (Davies and Morris 1993).

Source: **mouse** [Table 2.6.5.3A-Study R0100826] [Table 2.6.5.3B-Study RR-K00505-1]; **rat** [Table 2.6.5.3C-Study R01-0622] [Table 2.6.5.3E-Study R-7766]; **dog** [Table 2.6.5.3N-Study R-7703]; **monkey** [Table 2.6.5.3Q-Study R-7567] [Table 2.6.5.3R-Study R01-623-03]; **human** [Study A0108]

Table of Pharmacokinetic parameters of FTY720-P in blood after an intravenous or oral dose of [^{14}C]FTY720 in various species (Sponsor's)

	Mouse	**Rat**	**Monkey**	**Mouse**	**Rat**	**Rat**	**Rat**	**Dog**	**Monkey**	**Human**
Sex	male	male	male	male	male	male	male	male	male	male
Dose (mg/kg)	2.5	4	0.1	2.5	7.5	2.5	2.5	1.0	1.0	0.0545 [b]
Administration mode	i.v., sngl	i.v., sngl	i.v., sngl	p.o., sngl	p.o., sngl	p.o., sngl	p.o., 14 days	p.o., sngl	p.o., sngl	p.o., sngl
PK parameter: [$]										
T_{max} (h)	0.083 [a]	n.a.	1.0	8	24	8	8	8	8	7
C_{max} (ng/mL)	391 [a]	n.a.	7.09	161.6	58.2	256	453	56.2	28.9	1.37
C_{max} / dose (ng/mL)/(mg/kg)	157	-	70.9	64.6	437	103	181	56.2	28.9	25.1
AUC (ng·h/mL)	9532	20652	426	5889	26696	4650	8718	2309	1670	387
AUC / dose (ng·h/mL)/(mg/kg)	3813	5163	4262	2356	3559	1860	3487	2309	1670	7101
AUC interval	0-inf	0-168 h	0-inf	0-inf	0-168 h	0-24 h	0-24 h(ss) [e]	0-inf	0-inf	0-inf
FTY720/FTY720-P molar AUC ratio	1.4	2.7	1.0	1.5	3.6	2.4	2.7	1.3	1.1	0.54
Apparent terminal half-life $t_{1/2}$ (h)	26.1	41 [d]	65	21.6	20.5 [d]	n.a.	28.5	28.1	78	166
Accumulation factor (R_a)	2.1	3.0 [c]	4.4	1.9 [c]	1.8 [c]	n.a.	1.9	2.24 [c]	5.2 [c]	10.5 [c]
Study number	[R0100826]	[R01-0622]	[R01-623-03]	[R0100826]	[R01-0622]	[R0200921]	[R0200921]	[R0300770]	[R01-623-03]	[A2217]

$: to obtain µmol units, divide ng units by the molecular weight of FTY720-P (387.46). n.a.: not assessed. sngl: single dose.

a: first sampling time point.

b: human volunteers were dosed with 5 mg FTY720 hydrochloride (4.47 mg free base), mean body weight 82 kg.

c: calculated by: $R_a = 1/(1-\exp(-\ln 2/t_{1/2}\cdot\tau))$, τ = dosing interval (=24 h).

d: calculated from FTY720-P concentration data between 24 and 168 h obtained from metabolite profile analysis as given in report.

e: time period 0-24 h at steady state.

Source: **mouse** [Table 2.6.5.3A-Study R0100826]; **rat** [Table 2.6.5.3C-Study R01-0622] [Table 2.6.5.4C-Study R0200921]; **dog** [Table 2.6.5.3L-Study R0300770]; **monkey** [Table 2.6.5.3R-Study R01-623-03]; **human** [Study A2217]

5.1.2 Distribution

The Sponsor evaluated the distribution of FTY720 in blood of several animal species. The fraction of FTY720 in blood cells was approximately 90% in rat and dog and 86% in human. FTY720-P distribution was more variable with uptake into blood cells, at high concentrations (30-3000 ng/ml), of 54%, 44%, 33%, 13% and 17% for mouse, rat, monkey, dog and human, respectively; the values were lower at low concentrations

(0.2-2 ng/ml). In addition, for the primary metabolites M2 (hexanoic acid derivative) and M3 (butanoic acid derivative), the fraction in blood cells for M2 was 28%, ~0% and 11% in rat, dog and human blood, respectively and for M3 was 65%, 56% and 57% in rat, dog and human blood, respectively. Plasma protein binding was high for FTY720 (99.7-99.9%) and FTY720-P (98.3-99.6%) mouse, rat, dog, monkey and human.

Tissue distribution studies were performed in several species, including mice, rats and dogs. FTY720 was widely distributed into tissues in both rodents and dogs, consistent with the steady state volume of distribution observed (17.3, 21 and 7.6 l/kg for mice, rats and dogs, respectively).

Selected tissue T_{max}, C_{max} and $C_{168\ h}$ values were calculated after a single oral administration of 7.5 mg/kg [^{14}C]FTY720 to pigmented and albino rats. After multiple doses, accumulation of radioactivity occurred in several tissues. Accumulation in tissues after single and 14 days oral administration of 2.5 mg/kg [^{14}C]FTY720 to rats are summarized in the following table.

Table of organ pharmacokinetic parameters

Tissue	**Pigmented**				**Albino**	
	T_{max} (h)	**C_{max} (nM/g)**	**$C_{168\ h}$ (nM/g)**	**~$T_{½}$ (h)**	**$C_{168\ h}$ (nM/g)**	**Acc.**
Liver	8	67.14	0.84		0.76	1.7
Stomach (glandular)	8	66.13	1.02		1.00	1.6
Esophagus	8	55.35	0.60		0.55	3.3
Spleen	8	47.97	0.85		0.90	1.6
Adrenal cortex	8	47.54	1.18		0.86	1.7
Kidney cortex	8	47.24	0.88		1.00	1.7
Pituitary gland	24	42.15	3.44		8.88	2.4
Kidney medulla	8	42.09	0.99		1.24	1.3
Thyroid gland	8	34.02	1.14		0.85	2.2
Adrenal medulla	24	33.38	0.61		1.02	1.5
Brown fat	24	32.03	0.80		1.02	2.3
Lung	8	29.83	0.49		0.37	1.6
Bone marrow	24	18.57	0.40		0.33	1.4
Heart	8	17.44	0.46		0.44	1.2
Lymph nodes	8	14.91	0.72		0.53	1.5
Eye ocular membrane	24	14.05	3.56	160	0.49	---
Skin sub cutis	24	12.62	0.29		0.22	1.7
Spinal cord	72	6.96	6.05		4.81	7.0
Nerve	24	6.54	3.87	200	3.28	---
Skin epidermis	24	6.38	0.50	320	0.70	---
Brain	72	6.26	2.79	140	2.46	6.5
Epididymis	24	5.14	2.54		2.40	3.5
Blood	8	5.05	trace		trace	1.5
Skin dermis	24	4.46	0.47		0.31	---
Testes	72	3.51	3.14	460	2.40	7.0
White Fat	24	2.18	0.23	160	0.13	2.2

Acc. - accumulation (ratio between the concentrations observed at 24 hours after 14 daily doses and the concentrations observed at 24 hours after a single dose).

High uptake and accumulation of FTY720 into brain was observed in rats, dogs and monkeys. In rats, steady state brain concentrations were predicted, based on PK modeling, to be reached after 23 days of repeated daily oral administration, and were estimated to be 7.9-fold higher than after a single dose. Using similar modeling in dogs, predicted steady state brain concentrations after 36 days were increased 14.7-fold compared to that following an acute dose. For FTY720, the brain elimination half-lives were measured at 5.1, 8.15 and 22 days for rats, dogs, and monkeys, respectively. After 14 days of repeated doses of 7.5 mg/kg FTY720, at 8 hours post last-dose, the FTY720 brain-to-blood ratio was 27.7 and spinal cord-to-blood ratio was 11.3; ratios for the (S)-enantiomer of FTY720-P were 3.6 and 9.9.

A single oral dose of 7.5 mg/kg [^{14}C]FTY720 was administered to lactating rats on Day 10 after parturition. Milk and blood samples were collected up to 72 hours post-dose. FTY720 and metabolites were measured in milk up to 48 hours post-dose. The concentration ratio for FTY720 and FTY720-P increased with time, with milk/blood ratios at 0.25 hours of 0.27 and 0.11, respectively, and at 48 hours of 2.7 and 1.2, respectively. These data suggest that nursing infants would be exposed to FTY720 or FTY720-P. If these data are extrapolated to humans, the Sponsor states, "*the maximal dose of FTY720 ingested by the infant would amount to about 5 µg/kg which would be about 30% of the maternal dose of 18 µg/kg. This dose, together with the FTY720-P ingested with the milk, could lead to a pharmacologically significant exposure of the infant to FTY720 and FTY720-P*".

Table of FTY720 and metabolite blood and milk concentrations

	Rat Blood ($AUC_{0\text{-}48\ h}$)		**0.25 h (µmol/l)**		**1 h (µmol/l)**		**8 h (µmol/l)**		**24 h (µmol/l)**		**48 h (µmol/l)**	
	µM*h/l	%	Blood	Milk	Blood	Milk	Blood	Milk	Blood	Milk	Blood	Milk
[^{14}C]	99.9	100	0.44	0.16	1.32	0.56[b]	3.11	2.57[b]	2.36	3.91	0.97	1.57
FTY720	20.4	20.4	0.31	0.08	0.46	0.36	0.63	0.66	0.47	0.94	0.15	0.40
FTY720-P	42.9	42.9	0.07	0.01	0.44	0.09	1.35	0.36	1.04	1.84	0.40	0.47
Total	63.3	63.3	0.38	0.09	0.90	0.45	1.98	1.02	1.51	2.78	0.55	0.87
M1	0.1	0.1	0.01	-	0.02	-	nd	-	nd	-	nd	-
M2	1.2	1.2	0.01	0.01[c]	0.05	nd	0.05	0.40[c]	0.02	nd	0.00	nd
M3	17.7	17.8	0.01	0.03[c]	0.16	0.02	0.77	12.5[c]	0.34	0.14	0.12	0.05
M20	0.7	0.7	nd	-	nd	-	0.01	-	0.02	-	0.01	-
M23	1.2	1.2	nd	-	0.01	-	0.01	-	0.03	-	0.15	-

a – one value, b – two values, c – probably due to urine contamination, nd – not detected.

The placental transfer of FTY720 and metabolites was investigated in pregnant rats after a single oral dose of 0.45 mg/kg [^{14}C]FTY720 on Days 13 and 18 of gestation and in pregnant rabbits after a single oral dose of 5 mg/kg [^{14}C]FTY720 on Day 17 of gestation. In rats, T_{max} of total radioactivity in placenta (24 hours) and fetuses (48 hours) was delayed compared to the T_{max} of dams (8-24 hours). Fetal plasma exposure ($AUC_{0\text{-}48\ h}$) was similar to that in dams, and 6- and 3.8-fold lower than placenta exposure at gestation Days 13 and 18, respectively. In rabbits at 24 hours post-dose, radioactivity levels in the fetuses and amniotic fluids were approximately 4- and 12-fold lower, respectively, than in the dams' blood, while placenta levels were

approximately 2-fold higher. The circulating metabolite pattern in the fetuses was similar to that in the dams, with high levels of M3 and M4 and trace levels of M2.

Table of FTY720 and metabolite blood and fetus concentrations

	Rat (Gestation Day 18)				Rabbit			
	Dam Blood at 8 h		Fetus at 8 h		Dam Blood ($AUC_{0-24\ h}$)		Fetus	
	nmol/g	%	nmol/g	%	µM*h	%	pmol/g	%
[^{14}C]	0.278	100	0.165	100	19.332	100	193	100
FTY720	0.0260	9.35	0.0674	40.85	1.353	7.0	8	4.1
FTY720-P	0.1160	41.73	0.0390	23.64	0.988	11.8	8	3.9
Total	0.1420	51.08	0.1064	64.49	2.341	18.8	16	8.0
M1					0.204	1.1	nd	nd
M2	0.0033	1.19	0.0003	0.18	0.985	5.1	2	1.1
M3	0.0514	18.49	0.0030	1.82	7.057	36.5	55	28.4
M4	0.0078	2.81	0.0010	1.54	2.631	13.6	38	19.8

nd – not detected, * - values for mixture of the 2 enantiomers (however, FTY720-P, in fetuses at 8 h, represented exclusively the (S)-enantiomer), # - values for (S)-enantiomer.

5.1.3. Metabolism

The biotransformation of fingolimod in humans occurs by three main pathways:

(i) by reversible stereoselective phosphorylation to the (S)-enantiomer of fingolimod-P (AML629, the active moiety),

(ii) by oxidative biotransformation mainly via the cytochrome P450 4F2 isoenzyme and subsequent fatty acid-like degradation to inactive metabolites,

(iii) by formation of nonpolar ceramide analogs of fingolimod.

Following single oral administration of [^{14}C]fingolimod to humans (FTY720A2217), the major fingolimod-related components in blood, as judged from their contribution to the $AUC_{(0-816)b}$ of total radiolabeled components, are fingolimod itself (23.3%), fingolimod-P (10.3%), M3 (8.3%), M29 (8.9%) and M30 (7.3%).

As seen from the Sponsor's comments above, FTY720 is phosphorylated to the active moiety FTY720-P. There are 2 enantiomers of FTY720-P, the active (S)-enantiomer and the inactive (R)-enantiomer. The inactive (R)-enantiomer is not detected in blood or brain above the limit of detection (1-6% of total FTY720 in the sample). When [^{14}C]AML627 ((R)-enantiomer) was administered to rats, AML627 was rapidly de-phosphorylated to FTY720 and re-phosphorylated to the (S)-enantiomer, with only 10% of FTY720-P represented by AML627 after 1 hour post-administration. Further, there are no major human metabolites, as no metabolite is greater than 10% of the total radioactivity.

5.1.3.1 *In vitro*

In vitro biotransformation of FTY720 in human hepatocytes was primarily by phosphorylation to the active moiety, FTY720-P, however, no FTY720-P was observed in rat hepatocytes, possibly due to high phosphatase activity. Metabolism of the active moiety, FTY720-P, was first by de-phosphorylation, then oxidation to M1 (major in rats) and subsequent carboxylation to M2 and M3 (main in human). In rats, the principal enzyme involved in FTY720 metabolism is CYP4A1.

5.1.3.2. *In vivo*

In *in vivo* experiments in mice, FTY720 and FTY720-P were the major components in plasma. Major metabolites include M3 and M4 with traces levels of the nonpolar metabolites M28, M39 and M30, analogs of endogenous ceramides. In rat plasma, FTY720 and metabolites FTY720-P, M2, M3, M4, M5, M14, M20, M23, M24, M27, M27, M29 and M30 were detected. FTY720-P (50% of total radioactivity), FTY720 (18%), M3 (5.5%) and M23 (4.8%) had the highest plasma exposures ($AUC_{0\text{-}168\ h}$). Primary plasma metabolites after a single oral dose to dogs of FTY720 were M3 and PK3 (presumed FTY720-P), with the highest levels detected 6 and 24 hours post dose, respectively. Following i.v. administration to dogs, a high level of FTY720 was present at 5 minutes, but quickly decreased due to metabolism to primary metabolites FTY720-P and M3 with highest levels detected 1 and 24 hours post dose, respectively. Other metabolites, M1, M2 and M4, were produced more slowly, with highest values also at 24 hours. In a further dog study, FTY720 was metabolized mainly to FTY720-P (28%, of total radioactivity) and M3 (17%); FTY720 accounted for 12% of total radioactivity after 168 hours. Other circulating metabolites include M4, M2, M1 and M30 (1.8%). In the monkey study, FTY720 levels could not be calculated because of [^{14}C]FTY720 contamination. There was a high level of other metabolites (28.6 µM*h/l); however plasma exposures were greatest for FTY720-P (11.8 µM*h/l) and M3 (10.8 µM*h/l). Other circulating metabolites included M2 (4.1 µM*h/l), M30 (2.5 µM*h/l), M29 (0.9 µM*h/l) and M7 (0.4 µM*h/l). Metabolites M29 and M30 were produced very slowly in dog and monkey with C_{max}'s at 72 and 168 hours, respectively. In rat, dog and monkey, FTY720-P was detected exclusively as the active (S)-enantiomer.

Figure of rat metabolism of FTY720 (Sponsor's, page 34 and 35)

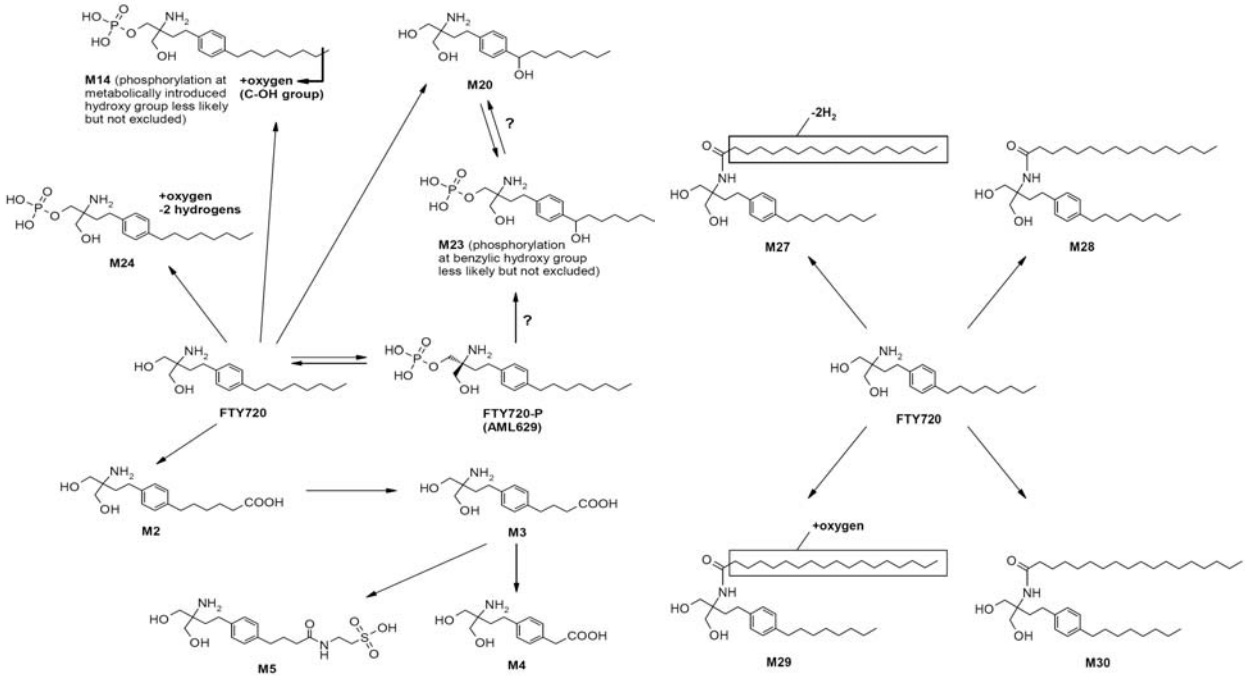


Table of plasma exposure after a single oral dose in mouse (2.5 mg/kg), rat (7.5 mg/kg), dog (1 mg/kg), monkey (3 mg/kg) and human (5 mg)

	Mouse ($AUC_{0\text{-}168\ h}$)[a]		Rat ($AUC_{0\text{-}168\ h}$)[b]		Dog ($AUC_{0\text{-}168\ h}$)[c]		Monkey ($AUC_{0\text{-}168\ h}$)[d]		Human ($AUC_{0\text{-}816\ h}$)[e]	
	µM*h/l	%	µM*h/l	%	µM*h/l	%	µM*h/l	%	µM*h/ml	%
[^{14}C]	54.8	100	142	100	21.5	100	59.1	100		
FTY720	12.4	22.6	25.2	17.8	2.6	12.3	9.0[?]	15.2	2.176	23.3
FTY720-P	24.9	45.5	70.4	49.7	5.9	27.6	11.8	20.2	0.965	10.3
Total	37.3	68.1	95.6	67.5	8.5	39.9	-	-	3.141	33.6
M2	0.04	0.08	0.63	0.45	0.6	2.6	4.1	6.9	0.14	1.5
M3	4.62	8.43	7.72	5.45	3.7	17.3	10.8	18.3	0.776	8.3
M29	0.27	0.50	0.9	0.64	-	-	0.9	1.5	0.834[#]	8.9
M30	0.65	1.18	1.62	1.14	0.4*	1.8	2.5	4.2	0.684[@]	7.3

a – Study № DMPK-r0100826, b – Study № DMPK-r0500354, c – Study № DMPK-r0500907, d – Study № DMPK-r0600988, e – Study № A2217 (5 mg).
***C_{max} = 168 hours. [#] C_{max} = various 36, 216, 480 hours. [@] C_{max} = 72 hours**

Table of plasma exposure to FTY720 and metabolites after oral dosing (Sponsor's)

Species	Human	Mouse	Rat	Rat	Rat	Rabbit	Dog	Monkey	Monkey
Dose (mg/kg)	0.0545 [a]	2.5	7.5	2.5	2.5	5.0	1.0	1.0	3.0
Administration mode, Frequency	p.o., single	p.o., single	p.o., single	p.o., rep., Day 1	p.o., rep., Day 14	p.o., single, Day 17 of gestation	p.o., single	p.o., single	p.o., single
Compound, Metabolite [b, e]	$AUC_{(0\text{-}816h)}$ [ng·h/mL]	$AUC_{(0\text{-}168h)}$ [ng·h/mL]	$AUC_{(0\text{-}168h)}$ [ng·h/mL]	$AUC_{(0\text{-}24h)}$ [ng·h/mL]	$AUC_{(312\text{-}336h)}$ [ng·h/mL]	$AUC_{(0\text{-}24h)}$ [ng·h/mL]	$AUC_{(0\text{-}168h)}$ [ng·h/mL]	$AUC_{(0\text{-}168h)}$ [ng·h/mL]	$AUC_{(0\text{-}168h)}$ [ng·h/mL]
FTY720	669	3813	5842	945	1718	416	799	1030	n.a.
FTY720-P	374	9648	26696	2925	6094	383	2286	1352	4572
M1	-	17	270	-	51	69	135	-	-
M2	43	12	588	88	89	305	186	430	1269
M3	218	1300	5430	656	626	1985	1041	1539	3039
M4	-	567	532	72	162	666	329	-	-
M5	-	206	466	66	213	-	-	-	-
M7	-	-	-	-	-	-	-	376	145
M10	-	-	740	-	-	-	-	-	-
M13	-	-	960	-	-	-	-	-	-
M14 [c]	-	-	2179	119 [c]	408 [c]	-	-	-	-
M20 [c]	-	-	-	96 [c]	327 [c]	-	-	-	-
M23	-	-	-	102	824	-	-	-	-
M24	-	-	-	64	219	-	-	-	-
M27	41	-	-	6	98	-	-	-	-
M28	25	497	-	8	108	-	-	-	-
M29	492	159	-	5	205	-	-	-	531
M30	393	373	-	16	530	-	-	-	1435
Reference:	[Study A2217]	[R0100826]	[R01-0622]	[R0200921]	[R0200921]	[R0200917]	[R0500907]	[R01-623-03]	[R0600988]

Footnotes to Table 11-15

-: not detected.

a: human volunteers were administered 5 mg [^{14}C]FTY720 hydrochloride (4.47 mg free base), mean body weight 82 kg.

b: for chemical structure and molecular weights of FTY720 and its metabolites see PT-Figure 12-9.

c: M14 and M20 co-eluted in the radio-chromatogram.

d: M15 is proposed to be mono-hydroxylated FTY720 at one of the methylene groups (pos. 1-7) of the octyl side chain of FTY720 [Table 2.6.5.10A-Study R0301153].

e: Metabolites M6, M8, M9, M11, M12, M15-M19, M22, M25 and M26 were not detected in the blood of any species including man.

Source:**mouse** [Table 2.6.5.9A-Study R0100826]; **rat** [Table 2.6.5.9C-Study R01-0622] [Table 2.6.5.9J-Study R0200921]; **rabbit** [Table 2.6.5.7C-Study R0200917]; **dog** [Table 2.6.5.9N-Study R0500907]; **monkey** [Table 2.6.5.9O-Study R01-623-03] [Table 2.6.5.9P-Study R0600988]; **human** [Study A2217];

Table of plasma exposure to FTY720 and metabolites compared to human (Sponsor's)

Species	Dose level	Dose (mg/kg/day)	Treatment duration	Exposure parameter	Compound / Metabolite [m] FTY720	FTY720-P	M2	M3	M29	M30
Human, HV [D2101]	therapeutic	0.018 [f]	7 days	AUCτ	140 [b]	75 [b]	20 [a]	50 [a]	197 [c]	149 [c]
Monkey, Cynomolgus	at NOAEL	< 1 [d, e]	52 wk	AUCτ [g]	< 660 [n]	< 1670	< 489	< 1702	< 334	< 1052
				ER	< 4.7	< 22	< 24	< 34	< 1.7	< 7.1
[971546]	at LOAEL	1 [d, e]	52 wk	AUCτ [g]	660 [n]	1670	489	1702	334	1052
				ER	4.7	22	24	34	1.7	7.1
Dog, Beagle	at NOAEL	0.01	26 wk	AUCτ [h]	10 [n]	24	2	12	n.d.	6
				ER	0.1	0.33	0.09	0.24	-	0.04
[R-7393]	at LOAEL	1	26 wk	AUCτ [h]	1351 [n]	2441	186	1210	n.d.	574
				ER	10	33	9.3	24	-	3.9
Rabbit [987117]	at NOAEL	0.5	2 wk	AUCτ [i]	16 [L]	38	30	199	n.a.	n.a.
				ER	0.11	0.51	1.5	4.0	-	-
	at LOAEL	1.5	2 wk	AUCτ [i]	57 [L]	115	91	596	n.a.	n.a.
				ER	0.41	1.5	4.6	12	-	-
Rat, SD [R-7219]	at NOAEL	0.3	26 wk	AUCτ [j]	107 [o]	731	11	75	25	64
				ER	0.76	9.8	0.53	1.5	0.12	0.43
	at LOAEL	10	26 wk	AUCτ [j]	8747 [L]	24377	356	2503	819	2119
				ER	62	325	18	50	4.2	14
Mouse, CD [991008]	at NOAEL	0.5	13 wk	AUCτ [k]	652 [L]	1953	2.5	263	47.2	95.3
				ER	4.7	26	0.12	5.3	0.24	0.64
	at LOAEL	5	13 wk	AUCτ [k]	6781 [L]	19528	25	2633	472	953
				ER	48	260	1.2	53	2.4	6.4

Exposure ratio ER animal vs human

In the rat 26 week (study №: PCS-r991052) and the monkey 39 week toxicity studies of FTY720, plasma levels of M2 and M3 were also assessed. Plasma levels for these 2 metabolites in humans after a single oral dose (5 mg) were 43 and 218 ng*h/ml (1.5% and 8.3% of total radioactivity), respectively (Study №: A2217). In the rat study, plasma levels (AUC_{0-24}) on Day 1-2 were 61 and 1,101.5 ng*h/ml, for M2 and M3, respectively, which is 1.3% and 24.2% of parent. In the monkey study, plasma levels (AUC_{0-24}) on Day 1-2 were 264 and 481 ng*h/ml, for M2 and M3, respectively, which is 28.7% and 52.3% of parent. Therefore, assuming linearity and extrapolating these human levels of metabolites after 5 mg administration to a 0.5 mg dose ($^1/_{10}^{th}$), plasma exposure to both metabolites was greater in both species than that measured in human plasma and, therefore, cover expected human levels. However, sufficient coverage of metabolites in nonclinical studies may not be the case for individuals with conditions (e.g., renal or hepatic impairment) that might result in higher levels of metabolite(s).

Table of toxicokinetic parameters after administration of 7.5 mg/kg to rats

	FTY720		M2				M3			
	Day 1-2	Wk 22	Day 1-2		Wk 22		Day 1-2		Wk 22	
Males										
C_{max}*	231	450	4.66	2.0%	4.46	1.0%	90	39.0%	81.2	18.0%
$AUC_{(0-24)}$#	4,439	9,342	61.5	1.4%	42	0.4%	1,240	27.9%	1,050	11.2%
Females										
C_{max}*	229	568	3.52	1.5%	8.08	1.4%	60.2	26.3%	72.1	12.7%
$AUC_{(0-24)}$#	4,664	9,772	60.5	1.3%	66.7	0.7%	963	20.6%	1,170	12.0%

*** ng/ml, # ng*h/ml, percentages are of the parent.**

Table of toxicokinetic parameters after administration of 3 mg/kg to monkeys

	FTY720			M2					M3					
	D 1-2	Wk 13	Wk 40	Day 1-2		Wk 13		Wk 40	Day 1-2		Wk 13		Wk 40	
Males														
C_{max}*	49.8	126	125	21.21	42.6%	31.36	24.8%	21.93	17.5%	38.78	77.9%	76.9	60.8%	47	37.1%
$AUC_{(0-24)}$#	886	2,473	2,536	256	28.9%	472	19.1%	328	12.9%	567	64.0%	1,316	53.2%	899	35.5%
Females														
C_{max}*	52.66	191	123	28.31	53.8%	65.24	34.1%	26.33	21.4%	28.35	53.8%	115	59.9%	67	54.1%
$AUC_{(0-24)}$#	952	3,491	2,543	272	28.6%	741	21.2%	366	14.4%	395	41.5%	1,596	45.7%	1,099	43.2%

*** ng/ml, # ng*h/ml, percentages are of the parent.**

5.1.4 Excretion

After oral and i.v. administration, the primary metabolites in rat urine were M3 and M5 and in rat feces were M1 and M2. There was no excretion of FTY720 or FTY720-P via the urine after either oral or i.v. administration. The primary metabolite in dog urine was M3, with M1, M2, M4, M5 and PK2 also detected. The primary metabolites in dog feces were M1, M2, M3 and FTY720-P, with FTY720, M4, M5 and PK1 also detected.

FTY720 brain exposure was assessed in rat and dog. Uptake studies with FTY720 and FTY720-P enantiomers, AML629 (S, active) and AML627 (R, inactive), demonstrated high brain penetration by all compounds. The main components in rat cerebral cortex and spinal cord after a single oral dose of FTY720 were FTY720 (28.4 and 13.1 nM/g, respectively) and FTY720-P (11.4 and 35.4 nM/g, respectively). Other metabolites detected included M28 (not detected and 0.1 nM/g, respectively) and M30 (0.3 and 0.2 nM/g, respectively). In cerebral cortex and spinal cord, FTY720-P was found to be exclusively the (S)-enantiomer.

Table of FTY720 blood and brain concentrations ($C_{24\,h}$)

Species	Duration	Dose (mg/kg)	Sampling time	Blood (ng/ml)		Brain (ng/ml)		Ratio	
				Male	Female	Male	Female	Male	Female
Rat	14 days	7.5	14 Days	311		8,517		28	
Dog	26 week	1	26 week	31.1	54.9	4,900	7,225	158	146
Monkey	52 week	1	52 week	28	27	5,675	3,750	206	139

5.1.5 Drug interactions

Studies indicated no drug-drug interactions between FTY720 and cyclosporine A, 42-O-(2-hydroxyethyl)-rapamycin (RAD001), sodium mycophenolate (ERL080) or methotrexate (MTX) in *in vivo* studies in Wistar rat or Beagle dog.

6 General Toxicology

6.1 Single-Dose Toxicity

Single oral dose studies were performed in mice, rats and dogs. The lethal dose in mice and M rats was 50 mg/kg and 25 mg/kg in F rats. FTY720 was tolerated up to 2000 mg/kg in dogs. Clinical signs in mice included signs of sedation, dyspnea, hunched posture, uncoordinated gait and tremors. In rats, signs included increased salivation immediately after dosing with other clinical signs occurring later: diarrhea, emaciation, decreased locomotion, bradypnea and hypothermia. The main signs in dog were vomiting and loose stools. The main target organ in the all species in the single dose studies was the lung, with histopathological findings in mice of lung discoloration, hemorrhage and a foamy fluid released from the bronchi. In rats, findings included accumulation of foamy cells in alveoli.

6.2 Repeat-Dose Toxicity

Study title: 13-Week oral dose-range finding study in mice

Study no.:	PCS-r991008
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland
Date of study initiation:	11 January 1999
GLP compliance:	Yes (Switzerland, 1986)
QA statement:	Yes (Dated: 14 Apr 2000)
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100%

Key Study Findings

- FTY720 was relatively well tolerated up to 5 mg/kg/day.
- Decreased lymphocyte counts, organ weights and histopathological findings of atrophy occurred in the lymphatic system.
- Increased heart weight was observed at all doses, but with no histopathological correlates.
- Histopathological findings included an increase in severity of kidney tubular basophilia and hyaline casts, increase in the number of mature neutrophilic granulocytes in 5 mg/kg/day male bone marrow and fibrinoid deposits in the lungs; lung weight was not assessed.
- Plasma exposure increased dose-proportionally in males and females with AUCs of 8,258 and 5,303 ng*h/ml at week 14 for 5 mg/kg/day males and females, respectively. The NOAEL was 0.5 mg/kg/day.

Methods

Doses:	0, 0.1, 0.5 and 5 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	10 ml/kg
Formulation/Vehicle:	(b) (4) graded water
Species/Strain:	Mouse/CD-1
Number/Sex/Group	
Main study:	10
Toxicokinetic study:	9
Age:	About 8 weeks at start of treatment
Weight	
Males:	31-42 g
Females:	24-31 g
Deviation from study protocol:	On Day 15 0.1 mg/kg/day animals (41-53, 61-79) were treated with 5 mg/kg/day. On Day 29 animal 51 did not receive the full 0.1 mg/kg dose

Observations and Results

Mortality

Observations were performed at least daily during pre-treatment and twice daily during treatment.

Two control animals died, one on Day 60. The other animal was not identified, but was probably from the toxicokinetic group.

Clinical Signs

Observations were performed at least daily during pre-treatment and twice daily during treatment.

There were no treatment-related changes.

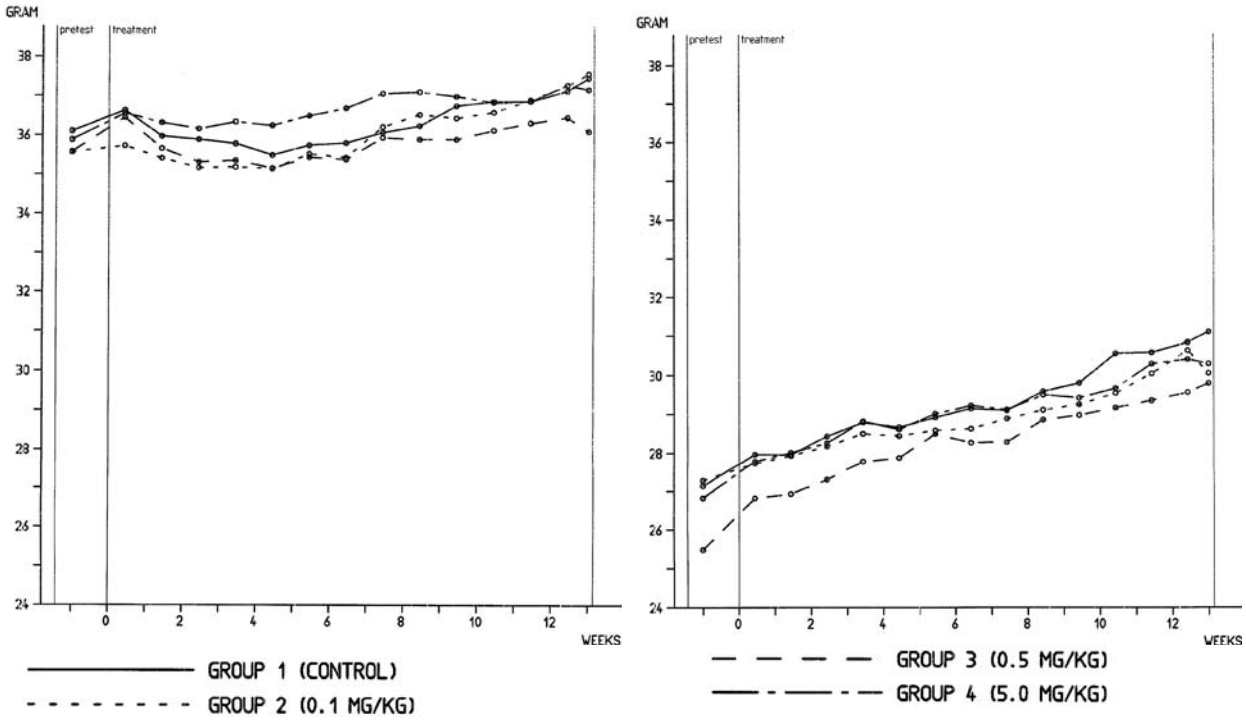
Body Weights

Body weights were recorded once during pre-treatment and daily during treatment.

No treatment-related change in body weight was recorded over the course of treatment.

Graphs of body weights (Sponsor's)

MALE **FEMALE**



Food Consumption

Food consumption was recorded once during pre-treatment and weekly during treatment.

There were no treatment-related changes in food consumption during the study.

Hematology

Blood samples were taken from the first 5 animals/dose/sex at the end of the study. The following parameters, listed on page 16 of the study report were assessed.

erythrocytes	mean corpuscular hemoglobin	reticulocytes
hematocrit	mean corpuscular hemoglobin concentration	thrombocytes
hemoglobin	mean corpuscular volume	white blood cell count**
inclusion bodies*	red cell distribution width	

* Counted if abnormalities were present

** Total and differential

At the HD, there was an increase in reticulocytes in M, but a decrease in F. FTY720 treated animals had decreased WBC counts, compared to controls (M: decreased by 76%, 67% and 47% at LD, MD and HD, respectively; F: decreased by 52%, 68% and 44% at LD, MD and HD, respectively). Lymphocytes were decreased by 69-88% and

basophils by 36-86% in all treated animals. Neutrophils and monocytes were increased in HD M and F.

Clinical Chemistry

Blood samples were taken from the last 5 animals/sex/dose at the end of the study. The following parameters were assessed:

alanine aminotransferase	cholinesterase	sodium
albumin	creatine kinase	total bilirubin
alkaline phosphatase	creatinine	total protein
aspartate aminotransferase	glucose	triglycerides
calcium	inorganic phosphorus	urea
chloride	magnesium	
cholesterol total	potassium	

In M, observations at the HD included increases in ALP (23%), urea (16%) and creatine (18%). Decreases in CK (61%, 57% and 70%) and choline (10%, 21% and 22%) were observed at the LD, MD and HD respectively. Decreases were also observed in triglycerides at the MD (19%) and HD (23%) and in total bilirubin (24%) at the HD.

F had increases in CK (118%, 39% and 34% at LD, MD and HD, respectively) and in urea (13%), creatine (11%), choline (16%) and triglycerides (29%) at the HD.

Gross Pathology

Gross examination was performed at necropsy, 1 day after last administration or as soon as possible after early death.

There were no gross findings.

Organ Weights

At necropsy the following organs were weighed:

brain	liver	testes*
heart	ovaries*	thymus
kidneys*	spleen	

* Paired organs weighed together

Changes in absolute organ weight were observed in heart, thymus, spleen and testes. Significant changes in relative organ weights occurred at the HD in M, with testes decreased by 15% and liver increased by 9% compared to control values. Heart weight was increased in M by 10%, 8% and 25% at the LD, MD and HD, respectively. In F, significant decreases in thymus (30%, 45% and 34%) and spleen (22%, 40% and 43%) relative weights were observed at all doses (LD, MD and HD) compared to control values and a dose-dependent increase was observed in relative heart weight (3%, 10% and 18% at LD, MD and HD, respectively). Lungs were not weighed.

Histopathology

At necropsy the following organs, as listed in the study report, were prepared for histopathology examination. Tissues were fixed in 10% formalin, except for testes, epididymides, prostate and seminal vesicles, which were fixed in Davidson's solution. Eyes with optic nerves, Harderian glands, lacrimal glands and pituitary gland were fixed in phosphate-buffered 10% formalin within the skull.

adrenals	knee joint	skeletal muscle
aorta	lacrimal glands	skin
bone marrow (in bone)	liver	spinal cord
brain	lungs	spleen
cecum	lymph node: bronchial	sternum
colon	lymph node: mandibular	stomach
duodenum	lymph node: mesenteric	testes
epididymides	mammary gland	thymus
esophagus	ovaries	thyroid with parathyroids
eyes with optic nerves	pancreas	tongue
gall bladder	peripheral nerves	trachea
Harderian glands	pituitary	urinary bladder
heart	prostate	uterus
ileum	rectum	vagina
jejunum	salivary glands	
kidneys	seminal vesicles	

The following tissues, as listed in the study report, from control and HD animals were processed histopathologically.

adrenals	lymph node: mandibular	spleen
bone marrow (sternum)	lymph node: mesenteric	stomach
epididymides	kidneys	testes
heart	liver with gall bladder	thymus
jejunum	lungs	thyroid with parathyroids
lymph node: bronchial	ovaries	

Adequate Battery: No, as the full battery was not examined and only control and HD animals were examined.

Peer Review: Peer review included of a review of the draft report, histological slides from at least 10% of the animals/organs.

The main organs showing changes after FTY720 administration were spleen, thymus, lymph nodes, kidneys, bone marrow and lungs. The findings in the lymphoid organs included minimal to marked cortical atrophy and medulla enlargement of the thymus, minimal to slight atrophy of the white pulp of the spleen and minimal to slight atrophy of the lymph nodes, especially of HD animals. Atrophy of primary lymphoid organs, thymus and spleen, is consistent with a pharmacological effect, as lymphocytes are sequestered in to secondary lymphoid organs and lymph nodes. Atrophy of lymph nodes was less severe and likely due to dying lymphocytes and cell debris. There was an increase in the severity of kidney tubular basophilia and hyaline casts in both sexes. M at the HD had a slight to moderate increase in the number of neutrophilic granulocytes in bone marrow. Lung findings were mainly in the HD animals, and consisted of minimal to slight cellular granulomatous reaction with fibrinoid deposits.

Summary table of major microscopic findings.

		MALE				FEMALE			
Tissue	**Finding**	**0**	**0.1**	**0.5**	**5**	**0**	**0.1**	**0.5**	**5**
Bone marrow - sternum	Neutrophilic gran. increase								
	slight:	0/9	0/10	0/10	3/10	0/10	0/10	0/10	0/10
	moderate:	0/9	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Myelofibrosis								
	minimal:	0/9	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Liver	Increased mitosis:								
	minimal:	0/9	0/0	0/0	1/10	0/10	0/0	0/0	0/10
Lungs	Granulomatous/Fibrinoid								
	minimal:	0/9	0/10	0/10	3/10	0/10	0/10	0/10	3/10
	slight:	0/9	0/10	0/10	2/10	0/10	0/10	0/10	1/10
	Foam cells								
	minimal:	0/9	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Inflammation								
	minimal:	1/9	0/10	0/10	0/10	0/10	0/10	2/10	1/10
	Mineralization								
	minimal:	0/9	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Kidneys	Tubular basophilia								
	minimal:	4/9	3/10	5/10	4/10	5/10	4/10	2/10	4/10
	slight:	0/9	1/10	0/10	3/10	1/10	0/10	1/10	0/10
	moderate:	0/9	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Hyaline cast								
	minimal:	2/9	4/10	6/10	7/10	2/10	6/10	4/10	6/10
	slight:	0/9	0/10	1/10	0/10	2/10	0/10	0/10	0/10
	Inflammation								
	minimal:	3/9	5/10	6/10	4/10	7/10	5/10	7/10	4/10
	slight:	2/9	1/10	0/10	4/10	1/10	2/10	0/10	0/10
	Mineralization								
	minimal:	0/9	0/10	0/10	0/10	0/10	1/10	0/10	0/10
Stomach	Cyst								
	minimal:	0/9	0/0	0/0	2/10	0/10	0/0	0/0	1/10
	Inflammation cell infiltration								
	minimal:	0/9	0/0	0/0	0/10	0/10	0/0	0/0	1/10
Spleen	Atrophy								
	minimal:	0/9	5/10	6/10	3/9	0/10	6/10	6/10	5/8
	slight:	0/9	0/10	0/10	5/9	0/10	0/10	2/10	3/8

Tissue	Finding	MALE 0	MALE 0.1	MALE 0.5	MALE 5	FEMALE 0	FEMALE 0.1	FEMALE 0.5	FEMALE 5
Spleen	Increase hematopoiesis								
	minimal:	0/9	5/10	4/10	4/9	6/10	4/10	3/10	3/8
	slight:	0/9	3/10	2/10	3/9	2/10	1/10	2/10	1/8
	moderate:	0/9	1/10	2/10	2/9	0/10	1/10	1/10	0/8
	Increase hemosiderosis								
	minimal:	0/9	1/10	0/10	0/9	4/10	4/10	5/10	4/8
	slight:	0/9	0/10	0/10	0/9	2/10	0/10	0/10	1/8
	moderate:	0/9	0/10	0/10	0/9	0/10	0/10	0/10	1/8
	Germ center development								
	minimal:	0/9	0/10	0/10	0/9	0/10	0/10	0/10	1/8
	Lymphocytolysis								
	minimal:	0/9	0/10	0/10	0/9	0/10	1/10	0/10	1/8
	Inflammation								
	minimal:	0/9	0/10	0/10	0/9	0/10	0/10	1/10	1/8
Thymus	Atrophy								
	minimal:	0/7	5/8	4/10	2/10	0/9	4/10	2/10	0/9
	slight:	0/7	0/8	2/10	5/10	0/9	1/10	4/10	0/9
	moderate:	0/7	0/8	0/10	3/10	0/9	0/10	1/10	7/9
	marked:	0/7	0/8	0/10	0/10	0/9	0/10	0/10	2/9
	Enlarged medulla								
	minimal:	0/7	3/8	1/10	1/10	0/9	4/10	4/10	0/9
	slight:	0/7	3/8	5/10	0/10	0/9	0/10	3/10	2/9
	moderate:	0/7	2/8	4/10	7/10	0/9	1/10	0/10	7/9
	marked:	0/7	0/8	0/10	2/10	0/9	0/10	0/10	0/9
	Lymphocytolysis								
	minimal:	0/7	0/8	0/10	3/10	3/9	1/10	0/10	3/9
	slight:	0/7	0/8	0/10	0/10	1/9	1/10	0/10	0/9
Bronchial Lymph Node	Germinal centers								
	minimal:	2/7	0/2	2/6	1/7	1/7	0/7	0/8	3/9
	slight:	0/7	0/2	0/6	0/7	0/7	0/7	0/8	0/9
	moderate:	0/7	0/2	0/6	1/7	0/7	0/7	0/8	0/9
	Lymphoid atrophy								
	minimal:	0/7	0/2	0/6	3/7	0/7	0/7	0/8	2/9
	Erythrophagocytosis								
	minimal:	0/7	0/2	0/6	1/7	0/7	0/7	0/8	2/9
	Pigmented macrophage								
	minimal:	1/7	1/2	1/6	3/7	1/7	3/7	2/8	3/9
	Inflammatory cell infiltration								
	minimal:	0/7	0/2	0/6	0/7	0/7	0/7	0/8	0/9
	slight:	0/7	0/2	0/6	0/7	0/7	0/7	0/8	0/9
	moderate:	0/7	0/2	0/6	1/7	0/7	0/7	0/8	0/9
Mandibular Lymph Node	Germinal centers								
	minimal:	4/7	1/5	1/4	2/7	3/7	2/4	1/7	0/5
	slight:	0/7	0/5	0/4	0/7	0/7	0/4	0/7	0/5
	moderate:	1/7	0/5	0/4	0/7	0/7	0/4	0/7	0/5
	Lymphoid atrophy								
	minimal:	0/7	0/5	0/4	2/7	0/7	0/4	0/7	0/5
	Lymphoid hyperplasia								
	minimal:	1/7	0/5	0/4	0/7	0/7	0/4	0/7	0/5
	Inflammatory cell infiltration								
	minimal:	0/7	0/5	0/4	0/7	0/7	0/4	0/7	0/5
	slight:	0/7	0/5	0/4	1/7	0/7	0/4	0/7	0/5

Tissue	Finding		MALE 0	MALE 0.1	MALE 0.5	MALE 5	FEMALE 0	FEMALE 0.1	FEMALE 0.5	FEMALE 5
Mesenteric Lymph Node	Germinal centers									
		minimal:	**3/9**	**2/9**	**4/8**	**5/7**	**4/9**	**4/8**	**6/10**	**4/9**
		slight:	**3/9**	**6/9**	**2/8**	**2/7**	**3/9**	**3/8**	**3/10**	**1/9**
		moderate:	**2/9**	**0/9**	**1/8**	**0/7**	**2/9**	**0/8**	**0/10**	**1/9**
	Lymphoid atrophy									
		minimal:	**0/9**	**4/9**	**4/8**	**4/7**	**0/9**	**1/8**	**5/10**	**5/9**
		slight:	**0/9**	**1/9**	**1/8**	**3/7**	**0/9**	**0/8**	**0/10**	**1/9**
	Lymphocytolysis									
		minimal:	**0/9**	**0/9**	**0/8**	**0/7**	**0/9**	**0/8**	**0/10**	**1/9**
	Pigment									
		minimal:	**0/9**	**0/9**	**0/8**	**0/7**	**0/9**	**0/8**	**1/10**	**0/9**
		slight:	**0/9**	**0/9**	**0/8**	**0/7**	**0/9**	**0/8**	**0/10**	**1/9**
Testes	Tubular atrophy									
		minimal:	**0/9**	**0/0**	**0/0**	**1/10**	**-**	**-**	**-**	**-**
Epididymis	Cellular debris									
		minimal:	**0/9**	**0/0**	**0/0**	**1/10**	**-**	**-**	**-**	**-**
Ovary	Cyst									
		present:	**-**	**-**	**-**	**-**	**0/10**	**0/0**	**1/1**	**1/10**

Toxicokinetics

Blood samples were taken on Weeks 5 and 14 at 1, 4, 7 and 24 hours post-dose. There were 2 animals per time point.

C_{max} and plasma exposure increased dose-proportionally in M and F with the plasma exposure slightly less in F. T_{max} was observed at 4-7 hours.

Table of toxicokinetic parameters (Sponsor's, page 317)

Period	week 5			week 14		
Dose [mg/kg/day]	0.1	0.5	5	0.1	0.5	5
Males						
t_{max} [h]	7	4	7	7	4	7
C_{max} [ng/mL]	5.93	31.7	365	7.21	41.1	428
C_{max}/dose(1)	59.3	63.4	73.0	72.1	82.2	85.6
AUC (0-24h) [h·ng/mL]	110	617	6854	145	745	8258
AUC (0-24h)/dose(2)	1098	1234	1371	1450	1489	1652
Females						
t_{max} [h]	7	4	7	7	4	7
C_{max} [ng/mL]	4.98	24.8	245	5.77	35.1	272
C_{max}/dose(1)	49.8	49.6	49.0	57.7	70.2	54.4
AUC (0-24h) [h·ng/mL]	86	428	4591	107	558	5303
AUC (0-24h)/dose(2)	856	855	918	1068	1116	1061

(1): [(ng/mL)/(mg/kg/day)]
(2): [(h·ng/mL)/(mg/kg/day)]

Stability and Homogeneity
FTY720 was stable and homogenously distributed.

Study title: Y-32720: One-month oral toxicity study in rats
Study no.: PCS-r6971
Study report location: EDR: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 10 December 1993
GLP compliance: Yes (b) (4) 1982)
QA statement: Yes (Dated 17 Jan 1995, amended 28 Aug 1998)
Drug, lot #, and % purity: Y-32720, Lot № I, 100.0%

Key Study Findings
- Decreased white blood cells and lymphocytes, lymphoid organ atrophy and decrease in spleen weight occurred.
- Increased heart and lung weight was observed, but with no histopathological correlates.
- No AUC values were calculated, but FTY720 plasma levels at 8 hours for 3 mg/kg on Day 1 were 36.6 and 47.5 ng/ml for male and female, respectively. An approximate 2-fold accumulation occurred during treatment.
- As there was an increase in organ weights at 3 mg/kg, the NOAEL is 0.3 mg/kg/day.

Methods

Doses:	0, 0.003, 0.1, 0.3 and 3 mg/kg (C, LD, MLD, MHD, HD)
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	4 ml/kg
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat/Crj:CD(SD))
Number/Sex/Group	
Main study:	10
4 week recovery:	6 for control, 0.3 and 3 mg/kg groups
Toxicokinetics:	4 for 0.01, 0.3 and 3 mg/kg groups
Age:	4 weeks
Weight	
Males:	119.2-137.2 g
Females:	105.8-120.6 g

Observations and Results

Mortality
There were no mortalities.

Clinical Signs
Observations were performed once pre-treatment and once or more post-dose during treatment and once daily during recovery.

The Sponsor stated that there was "*no clinical sign related to treatment*"; however, no data were submitted.

Body Weights
Body weights were recorded pre-treatment, twice weekly during treatment and once weekly during recovery.

There was a slight decrease in body weight at the HD, with weight gain over 4 weeks of 91.2% and 96.3% of control weight gain for M and F, respectively. Over the recovery period, weight gains were 92.3% and 100.4% of control weight gain for M and F at the HD, respectively, suggesting no recovery for M.

Food Consumption
Food consumption was determined for cages of animals on the first day of treatment and then weekly during treatment and recovery.

There was a 20% decrease in food consumption in M at the HD over the first week of treatment; otherwise, there were no treatment-related effects.

Ophthalmoscopy
Examinations of all main study animals were performed at Weeks 3-4 during treatment and Week 4 of recovery.

No ophthalmoscopy data were submitted. It was reported that there were no ophthalmologic findings.

Hematology
Blood samples were taken from all animals from the jugular vein at the end of the study. The following parameters were assessed: RBC and WBC counts, platelets, HGB and HCT.

At the HD, there was an increase in platelets in M and a decrease in RBCs, with an increase in MCH and MCHC in 1 F. WBC and lymphocyte counts were decreased in all groups, statistically significant in the MHD and HD dose groups with decreases of 70-87%. Monocytes were decreased by 48-65% in MHD and HD animals. Decreased APTT was observed in MHD F. Platelet and RBC changes were reversed after 4 weeks of recovery. There was some recovery of WBC and lymphocyte counts, with levels in HD animals decreased by 11-25% compared to control values at the end of the recovery period.

Clinical Chemistry
Blood samples were taken from all animals from the abdominal vein at the end of the study. The following parameters were assessed: GOT, GPT, ALP, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

In HD M, there was a statistically significant decrease in GOT (18%), total bilirubin (33%), β-globulin (11%), and an increase in albumin (4%), α_2-globulin (19%), and A/G ratio. In HD F, statistically significant changes included a decrease in protein (7%), and increases in glucose (25%), phospholipids (19%), albumin (8%), and A/G ratio. The changes reversed after the 4 week recovery period.

Urinalysis
Urine samples were taken from all main study animals at Weeks 3-4 during treatment and after Week 4 of recovery.

There was a slight decrease in the urinary pH that was still evident in F after the recovery period. Otherwise, there were no treatment-related changes.

Gross Pathology
Gross pathology was assessed in all animals.

Small spleens were observed in MHD and HD animals, with 2 M at the HD also having a dark red spleen. Other macroscopic observations were observed in the liver, kidney, adrenals and submandibular glands, mainly in M, but usually only 1 animal was affected.

Organ Weights
At necropsy, the following organs were weighed: brain, heart, lungs, liver, kidneys, spleen, submandibular glands and testes. The following organs were fixed in 10% formalin and weighed: pituitary, adrenals, thymus, thyroid glands, seminal vesicles, prostate, ovary and uterus.

A significant decrease in mean spleen weight occurred at the MHD and HD (M: 30% and 35%, respectively; F: 27% and 32%, respectively. In HD F, heart and lungs were significant increased by 5% and 13%, respectively; in MHD and HD F, ovary weights were significantly decreased by 13% and 18%, respectively. After recovery, MHD and HD spleen weights recovered, spleen weight of M at the HD only partial recovered. Heart, lung and ovary weight changes observed in treated F were also reversed.

Histopathology
The following organs were prepared for histopathology examination: liver, kidneys, lungs, heart, spleen, pancreas, brain, spinal cord, adrenals, thyroid glands, pituitary, thymus, submandibular lymph node, mesenchymal lymph node, femur bone, parotid glands, submandibular glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, urinary bladder, aorta, epididymis, seminal vesicles,

prostate, ovary, uterus, vagina, mammary gland, skin, skeletal muscle, tongue and sternum. Tissues were fixed in 10% formalin, except for testes, epididymis, Harderian glands and eye balls were fixed in Bouin's solution.

Adequate Battery: Yes
Peer Review: No

Histopathological observations included lymphoid atrophy in spleen, thymus and lymph nodes at the end of the dosing period, but not observed in recovery animals. Slight kidney tubular regeneration occurred in M at the HD and F at the MHD and interstitial fibrosis in M at the MHD and F at the HD, with no observations in recovery animals. An acinar cell adenoma in the pancreas was observed in 1 LD animal.

Summary of major male microscopic findings

		Main					Recovery		
Tissue	**Finding**	**0**	**0.003**	**0.01**	**0.3**	**3**	**0**	**0.3**	**3**
Heart	Myocarditis								
	very slight:	**0/10**	**1/10**	**0/10**	**0/10**	**0/10**	**0/6**	**1/6**	**1/6**
	slight:	**1/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/6**	**0/6**	**0/6**
Kidneys	Tubular regeneration								
	very slight:	**0/10**	**0/10**	**0/10**	**0/10**	**1/10**	**0/6**	**0/6**	**0/6**
	Interstitial fibrosis								
	very slight:	**0/10**	**0/10**	**0/10**	**1/10**	**0/10**	**0/6**	**0/6**	**0/6**
Spleen	White pulp atrophy								
	very slight:	**0/10**	**0/10**	**0/10**	**8/10**	**7/10**	**0/6**	**0/6**	**0/6**
	slight:	**0/10**	**0/10**	**0/10**	**2/10**	**3/10**	**0/6**	**0/6**	**0/6**
Thymus	Atrophy of cortex								
	very slight:	**0/10**	**0/10**	**0/10**	**10/10**	**10/10**	**0/6**	**0/6**	**0/6**
	Enlarged medulla								
	very slight:	**0/10**	**0/10**	**0/10**	**2/10**	**0/10**	**0/6**	**0/6**	**0/6**
	slight:	**0/10**	**0/10**	**0/10**	**8/10**	**10/10**	**0/6**	**0/6**	**0/6**
Mesenteric Lymph Node	Atrophy								
	very slight:	**0/10**	**0/10**	**0/10**	**5/10**	**6/10**	**0/6**	**0/6**	**0/6**
Submandibular Lymph Node	Atrophy								
	very slight:	**0/10**	**0/10**	**0/10**	**6/10**	**5/10**	**0/6**	**0/6**	**0/6**
Pituitary gland	Rathke's remnant								
	very slight:	**0/10**	**1/10**	**1/10**	**1/10**	**0/10**	**0/6**	**0/6**	**0/6**

Summary of major female microscopic findings

		Main					Recovery		
Tissue	**Finding**	**0**	**0.003**	**0.01**	**0.3**	**3**	**0**	**0.3**	**3**
Heart	Myocarditis								
	very slight:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**1/6**	**0/6**	**1/6**
	slight:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/6**	**0/6**	**0/6**
Kidneys	Tubular regeneration								
	very slight:	**0/10**	**0/10**	**0/10**	**1/10**	**0/10**	**0/6**	**0/6**	**0/6**
	Interstitial fibrosis								
	very slight:	**0/10**	**0/10**	**0/10**	**0/10**	**1/10**	**0/6**	**0/6**	**0/6**

Tissue	Finding	Main					Recovery		
		0	0.003	0.01	0.3	3	0	0.3	3
Kidneys	Dilatation								
	very slight:	0/10	0/10	0/10	0/10	0/10	0/6	0/6	0/6
	slight:	0/10	0/10	0/10	0/10	0/10	0/6	0/6	0/6
	moderate:	0/10	0/10	1/10	0/10	0/10	0/6	0/6	0/6
Spleen	White pulp atrophy								
	very slight:	0/10	0/10	0/10	10/10	7/10	0/6	0/6	0/6
	slight:	0/10	0/10	0/10	0/10	3/10	0/6	0/6	0/6
Thymus	Atrophy of cortex								
	very slight:	0/10	0/10	0/10	10/10	10/10	0/6	0/6	0/6
	Enlarged medulla								
	very slight:	0/10	0/10	0/10	7/10	3/10	0/6	0/6	0/6
	slight:	0/10	0/10	0/10	3/10	7/10	0/6	0/6	0/6
Mesenteric Lymph Node	Atrophy								
	very slight:	0/10	0/10	0/10	1/10	5/10	0/6	0/6	0/6
Submandibular Lymph Node	Atrophy								
	very slight:	0/10	0/10	0/10	3/10	1/10	0/6	0/6	0/6

Bone marrow smears

At autopsy bone marrow was harvested from femurs of each animal and stained with Light-Giemsa.

Increases in the percent of basophilic erythroblasts and myeloblast were observed in MLD, MHD and HD M, but with no effect on PCEs or premyelocytes.

Toxicokinetics

Blood samples were taken from 4 M and 4 F from MLD, MHD and HD groups on Days 1, 14 and the last day of administration at 8 and 24 hours post-dose.

No general sex differences in plasma levels at 8 and 24 hours post-dose were observed. At 8 hours post-dose, plasma levels increased dose-proportionally between MHD and HD doses and accumulation occurred over the course of treatment.

Table of Blood concentration of FTY720

Sex	Dose (mg/kg)	Day 1		Day 14		Day 28	
		8 hour	24 hour	8 hour	24 hour	8 hour	24 hour
Male	0.01	0.4	0.2	0.5	0.2	0.2	0.2
(ng/ml)	0.3	3.0	0.6	5.3	1.7	5.6	2.3
	3	36.6	14.7	55.3	27.3	90.8	35.2
Female	0.01	0.4	0.1	0.6	0.5	0.3	0.2
(ng/ml)	0.3	4.3	0.9	6.2	2.9	6.8	3.1
	3	47.5	15.6	63.7	36.2	85	44.5

Study title: Y-32720: One-month oral toxicity study in rats (additional high-dose study)

Study no.:	PCS-r7071
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 April 1994
GLP compliance:	Yes (b) (4), 1982)
QA statement:	Yes (Dated: 2 Aug 1995, amended 28 Aug 1998)
Drug, lot #, and % purity:	Y-32720, Lot № I, 100.0%

Key Study Findings

- Deaths occurred at 30 and 60 mg/kg/day, due to poor health. Marked decreases in body weight resulted in early sacrifice of all remaining 60 mg/kg/day animals on Day 10.
- Histopathological observations in 10 and 30 mg/kg/day animals included atrophy of the lymphoid organs, heart myocarditis, stomach edema and cell infiltration, macrophage infiltration of the lungs and pituitary gland vacuolation and atrophy.
- No AUC values were calculated, but FTY720 plasma levels at 8 hours on Day 1 were 363.5 and 251.3 ng/ml for 60 mg/kg/day males and females, respectively and accumulation occurred.
- A NOAEL could not be determined, as animals administered 10 mg/kg/day had large (~20%) decreases in body weight and increases in heart and lung weights.

Methods

Doses:	0, 10, 30 and 60 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	4 ml/kg
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat/Crj:CD(SD)
Number/Sex/Group	
Main study:	10
4 week recovery:	6 for 0, 30 and 60 mg/kg groups
Toxicokinetics:	4 for 10, 30 and 60 mg/kg groups
Age:	4 weeks
Weight	
Males:	134.6-155.8 g
Females:	108.5-129.2 g

Observations and Results

Mortality

As 5 M and 4 F at the HD were found dead by Day 10 and poor health and marked decreases in body weight were observed in the remaining HD animals; the remaining HD animals were sacrificed on Day 10. At the MD (main study, recovery and TK), 1 M

died on Day 6 and 2 F died on Days 5 and 28 with signs of emaciation, piloerection and breathing associated with rales.

Clinical Signs
Observations were performed once pre-treatment and at least once after each dose during treatment and once daily during recovery.

Occasional emaciation was observed in M at the MD and in 1 F at the MD that also had piloerection. There were sporadic rales in 4 M and 5 F at the MD. A dose-dependent increase in salivation was observed in LD and MD animals from Day 2, which was reversed during the recovery period. In the HD group prior to Day 10, there was tachypnea in 1 M and 1 F, rales in 3 M and 10 F, and emaciation in 8 F; all animals had increased salivation.

Body Weights
Body weights were recorded prior to treatment, twice weekly during treatment and once weekly during recovery.

There was a dose-dependent decrease in body weight gain. On Day 3, decreases compared to control weight gain were for M, 24%, 85% and 117% and for F, 33%, 95% and 162% at the LD, MD and HD, respectively. At the end of treatment weight gains were decreased by 17% and 26% in LD and MD M, respectively, and 19% and 24% in LD and MD F, respectively. The effect on weight gain was reversed in MD animals after 4 weeks recovery.

Food Consumption
Food consumption was determined for pairs of animals once pre-treatment and weekly during treatment and recovery.

In M, a 6-8% and 17-14% decrease in food consumption at the LD and MD, respectively occurred. Food consumption in the first week of treatment was decreased in F by 4-6% and 8-9% at the LD and MD, respectively. The decrease in food consumption continued during the recovery period for M and F.

Ophthalmoscopy
Examinations of all main study animals occurred at Weeks 3-4 during treatment and Week 4 of recovery.

No ophthalmoscopy data were submitted. It was reported that there were no ophthalmic findings.

Hematology
Blood samples were taken from all animals at the end of the study. The following parameters were assessed: erythrocyte, leukocyte and platelet counts, HGB and HCT, MCV, MCH, MCHC, reticulocytes, PT and APPT.

In M, statistically significant increases in RBCs, HGB, HCT and MCHC were observed at the MD. WBC and lymphocyte counts were decrease at all doses by 48-71% and 80-88%, respectively. Neutrophils were increased dose-dependently (44-65% and 111-150%) in animals at the LD and MD. There was recovery of the RBC parameters, but a decrease in WBC and lymphocyte counts was still evident (46-66% and 57-73% in M and F at the MD, respectively)

Clinical Chemistry
Blood samples were taken from all animals at the end of the study. The following parameters were assessed: GOT, GPT, ALP, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

Findings consisted of the following: In M, there were statistically significant increases in total cholesterol and decreases in total protein, β-globulin and γ-globulin at the LD. At the MD, BUN, albumin, and A/G were increased, whereas ALP, total protein, and α_1-globulin were decreased. These changes were not evident at the end of the recovery period.

In F, statistically significant changes included increases in potassium and chloride and decreases in glucose, calcium, total protein, and alkaline phosphatase at the LD, increases in chloride, total cholesterol, GOT, BUN, triglycerides, phosphorus, and A/G and decreases in total protein and alkaline phosphatase at the MD. Increases in GOT and decreases in total protein at the end of the recovery period.

Urinalysis
Urine samples were taken from all main study animals at Week 4 during treatment and at Week 4 of recovery.

Decreased urine volumes were observed in both M and F with resultant increases in specific gravity. Other changes included increased ion levels (sodium, potassium and chloride) and a slight increase in protein.

Gross Pathology
Gross pathology was assessed in all animals.

Small lymphoid organs were observed in animals at the LD and MD. Hepatization was observed in 2 MD F, stomach wall thickening in 2 MD M and decreased adipose tissue in all F dose groups. A dark red color was observed in lungs from dead animals.

Organ Weights
At necropsy, the following organs were weighed: brain, heart, lungs, liver, kidneys, spleen, submandibular glands and testes. The following organs were fixed in 10% formalin and weighed: hypophysis, adrenals, thymus, thyroid glands, seminal vesicles, prostate, ovary and uterus.

In M at the LD and MD, there were dose-dependent increases in relative weights of liver (7% and 13%), lungs (20% and 32%), heart (9% and 14%), testes (14% and 22%) and brain (14% and 17%) and decreases in relative weights of thymus (6% and 35%), spleen (33-39%) and prostate (29%). In recovery M at the MD, there was still evidence of changes in lung (12%), testes (10%), brain (10%), thymus (40%) and spleen (19%) weight.

In F at the LD and MD, there were dose-dependent increases in relative weights of liver (5% and 14%), lungs (29% and 44%), heart (14% and 16%) and brain (8% and 16%) and decreases in relative weights of spleen (29% and 44%). In F at the MD, thymus (42%) and ovary (16%) relative weights were decreased. In recovery F at the MD, increased lung (26%) and heart (6%) weights and decreased spleen (17%) weight were still observed.

In M and F at the HD, there were increases in the relative weights of liver (23% and 28%), lung (71% and 88%), heart (31% and 22%), kidneys (29% and 28%), adrenals (59% and 32%), testes (63%) and brain (121% and 85%), and decreases in thymus (29% and 54%), spleen (17% and 14%), prostate (29%), ovary (16%) and uterus (35%).

Histopathology

At necropsy the following organs were prepared for histopathology examination: liver, kidneys, lungs, heart, spleen, pancreas, brain, spinal cord, adrenals, thyroid glands, hypophysis, thymus, submandibular lymph node, mesenchymal lymph node, femur bone, parotid glands, submandibular glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, urinary bladder, aorta, epididymis, seminal vesicles, prostate, ovary, uterus, vagina, mammary gland, skin, skeletal muscle, tongue and sternum. Tissues were fixed in 10% formalin, except for testes, epididymis, Harderian glands and eye balls, which were fixed in Bouin's solution. Sections of the hypophysis were evaluated in 5 animals per sex per group using Azan stain.

Adequate Battery: Yes
Peer Review: No.

Histopathological observations included lymphoid atrophy in spleen, thymus and lymph nodes, which was less severe in recovery animals. Other observations included heart myocarditis, stomach edema and cell infiltration, macrophage infiltration of the lungs and pituitary gland vacuolation and atrophy at the MD. There were few observations in recovery animals.

In main study animals that died or were sacrificed early, atrophy of the lymphoid organs was detected. In addition, the 2 MD (1 M and 1 F) animals had findings in the lungs and pituitary gland. Lungs findings included slight atelectasis, alveolar collapse or fluid consolidation, infiltration of alveolar macrophages and pneumonia. In the F, there was slight vacuolation and atrophy in pituitary gland. Findings in animals at the HD included slight vascular wall thickening and moderate myocardial necrosis of the heart, and slight

edema, slight to moderate infiltration of alveolar macrophages and slight to marked pneumonia of the lungs.

Summary of major microscopic findings

		MALE					FEMALE				
		Main			Rec		Main			Rec	
Tissue	**Findings**	**0**	**10**	**30**	**0**	**30**	**0**	**10**	**30**	**0**	**30**
Heart	Myocarditis										
	slight:	0/10	0/10	1/9	0/6	0/6	0/10	0/10	1/9	0/6	0/6
Lungs	Macrophages infiltration										
	slight:	0/10	0/10	9/9	0/6	3/6	0/10	0/10	7/9	0/6	1/6
	Edema										
	slight:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	1/9	0/6	0/6
Stomach	Edema										
	slight:	0/10	0/10	5/9	0/6	0/6	0/10	0/10	2/9	0/6	0/6
	Cell infiltration										
	slight:	0/10	0/10	3/9	0/6	1/6	0/10	0/10	2/9	0/6	0/6
Spleen	White pulp atrophy										
	slight:	0/10	0/10	0/9	0/6	3/6	0/10	0/10	0/9	0/6	5/6
	moderate:	0/10	10/10	9/9	0/6	0/6	0/10	9/10	8/9	0/6	0/6
	marked:	0/10	0/10	0/9	0/6	0/6	0/10	1/10	1/9	0/6	0/6
	Extra. hematopoiesis										
	Slight:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	0/9	0/6	1/6
Thymus	Atrophy of cortex										
	slight:	0/10	10/10	0/9	0/6	6/6	0/10	10/10	0/9	0/6	0/6
	moderate:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	1/9	0/6	2/6
	marked:	0/10	0/10	9/9	0/6	0/6	0/10	0/10	8/9	0/6	4/6
	Enlarged medulla										
	slight:	0/10	2/10	0/9	0/6	0/6	0/10	0/10	0/9	0/6	0/6
	moderate:	0/10	8/10	0/9	0/6	0/6	0/10	10/10	0/9	0/6	0/6
Thymus	marked:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	0/9	0/6	0/6
Mesenteric	Atrophy										
Lymph Node	slight:	0/10	8/10	4/9	0/6	2/6	0/10	9/10	6/9	0/6	1/6
	moderate:	0/10	2/10	5/9	0/6	0/6	0/10	1/10	3/9	0/6	0/6
Submandibular	Atrophy										
Lymph Node	slight:	0/10	1/10	2/9	0/6	0/6	0/10	0/10	0/9	0/6	0/6
Adrenal glands	Calcification										
	slight:	0/10	0/10	0/9	0/6	0/6	0/10	6/10	2/9	0/6	0/6
Thyroid gland	Ultimobranchial body										
	slight:	0/10	0/10	0/9	1/6	0/6	0/10	0/10	3/9	0/6	0/6
	moderate:	1/10	4/10	3/9	0/6	0/6	0/10	1/10	1/9	0/6	0/6
	Atrophy										
	slight:	0/10	0/10	0/9	0/6	0/6	1/10	0/10	1/9	1/6	1/6
	Moderate:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	1/9	0/6	0/6
Pituitary	Vacuolation										
	slight:	0/10	0/10	4/9	0/6	0/6	0/10	0/10	3/9	0/6	0/6
	Atrophy										
	slight:	0/10	0/10	0/9	0/6	0/6	0/10	0/10	2/9	0/6	0/6
Prostate	Interstitial cell infiltration										
	slight:	1/10	3/10	3/9	2/6	1/6	-	-	-	-	-
	moderate:	0/10	1/10	0/9	0/6	0/6	-	-	-	-	-
	marked:	1/10	0/10	0/9	0/6	0/6	-	-	-	-	-

Bone marrow smears
At autopsy, bone marrow was harvested from femurs of each animal and stained with Light-Giemsa.

An increase in metamyelocytes was observed in F at the MD.

Toxicokinetics
Blood samples were taken from 4/sex from LD, MD and HD groups on Days 1, 14 and last day of dosing, at 8 and 24 hours post dose.

No general sex differences in plasma levels at 8 and 24 hours post-dose were observed. At 8 hours post-dose, plasma levels increased dose-proportionally and accumulation occurred over the course of treatment.

Table of toxicokinetic parameters (Sponsor's)

Sex	Dose (mg/kg)		0day		14day		28day	
			8hr	24hr	8hr	24hr	8hr	24hr
Male	10	N	4	4	4	4	4	4
		mean	141.5	97.4	178.1	91.9	331.3	219.7
		S.D.	19.0	18.0	17.5	10.4	50.7	38.3
	30	N	4	4	4	4	4	4
		mean	207.1	299.5	542.9	274.0	948.0	567.2
		S.D.	72.0	53.6	99.8	101.7	110.8	201.1
	60	N	4	4				
		mean	363.5	502.3				
		S.D.	78.5	117.6				
Female	10	N	4	4	4	4	4	4
		mean	148.5	93.6	181.5	117.2	381.9	182.7
		S.D.	44.2	9.5	24.4	20.7	42.1	13.4
	30	N	4	4	3	3	3	3
		mean	216.1	244.4	480.7	290.0	974.5	648.2
		S.D.	121.3	47.1	45.7	25.4	55.2	68.5
	60	N	3	3				
		mean	251.3	439.0				
		S.D.	42.2	132.7				

(unit:ng/ml)

Study title: FTY720: 13-Week oral dose-range finding study in rats

Study no.:	PCS-r991007
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	Novartis Pharma AG Basel Switzerland
Date of study initiation:	3 February 1999
GLP compliance:	Yes (Switzerland, 1986)
QA statement:	Yes (Dated: 2 Feb 2000, amended 20 Jan 2009)
Drug, lot #, and % purity:	Y-32720, Lot № 98905, 100%

Key Study Findings

- No deaths or clinical signs were observed.
- Findings included decreased white blood cells and lymphocytes, and lymphoid organ atrophy.
- The NOAEL was less than 0.1 mg/kg/day, as lung histopathological findings and increases in lung and heart weights were observed at all doses.

Methods

Doses:	0, 0.1, 0.5 and 5 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	(b) (4) water
Species/Strain:	Rat/CR/Wiga Han:Wistar
Number/Sex/Group	
Main study:	10
Age:	8 weeks, at start of treatment
Weight	
Males:	193-247 g
Females:	147-179 g

Observations and Results

Mortality

Observations were performed daily pre-treatment and at least twice daily during treatment.

There were no deaths.

Clinical Signs

Observations were performed daily pre-treatment and at least twice daily during treatment.

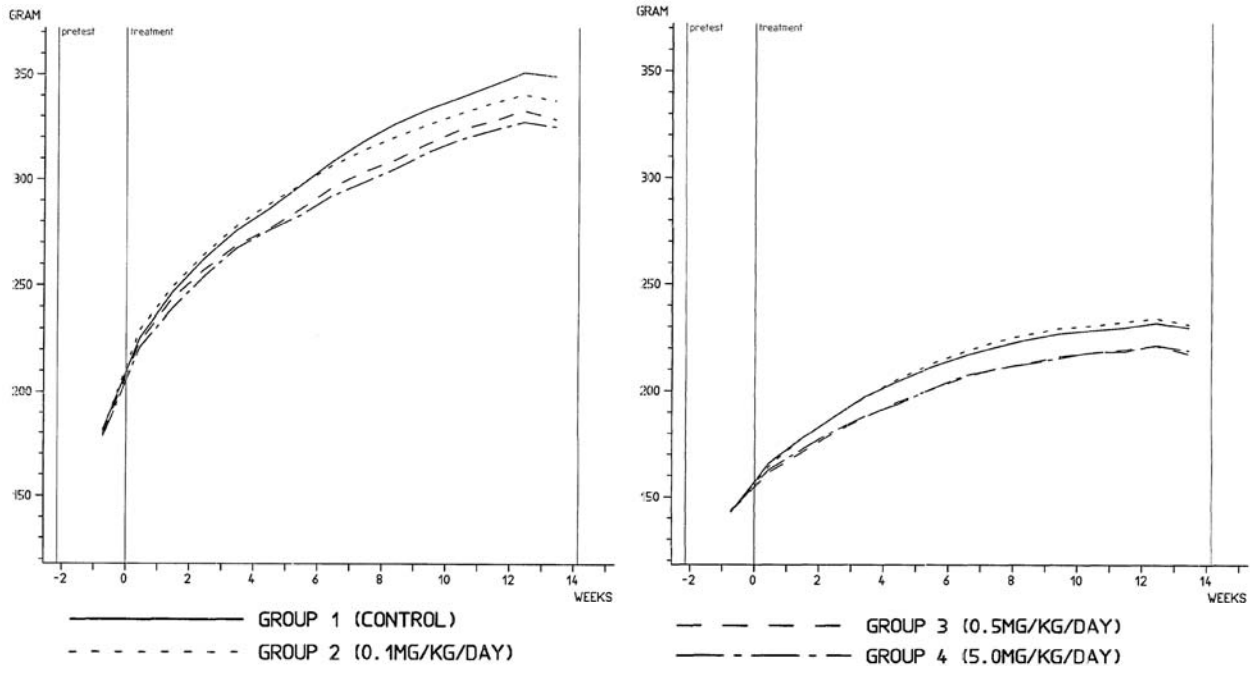
There were no treatment-related changes in clinical signs.

Body Weights
Body weights were recorded once prior to treatment and weekly during treatment.

There was a slight, dose-dependent decrease in total body weight gain over the course of treatment in M at the LD, MD and HD (3.5%, 6.0% and 6.9%, respectively). The decrease in weight gain was evident from the beginning of treatment at the HD. Total body weight gain was decreased in F at the MD and HD (5.2% and 4.4%, respectively).

Graphs of body weights (Sponsor's)

MALE **FEMALE**



Food Consumption
Food consumption was determined for cages once pre-treatment and weekly during treatment.

There were no differences in food consumption.

Hematology
Blood samples were taken at pre-treatment and at the end of the study. The following parameters were assessed:

erythrocytes	mean corpuscular hemoglobin	reticulocytes
inclusion bodies*	mean corpuscular hemoglobin concentration	thrombocytes
hematocrit	mean corpuscular volume	white blood cell count**
hemoglobin	red cell distribution width	

* Counted if abnormalities were present

** Total and differential

By Week 13 there was a dose-dependent increase in reticulocytes in M (16% and 22% at MD and HD, respectively) and F (13% and 23% at MD and HD, respectively), and a decrease in WBC counts and lymphocyte levels that were reduced by 58-80% and 83-92% of pre-treatment levels, respectively.

Clinical Chemistry

Blood samples were taken at pre-treatment and at the end of the study. The following parameters were assessed:

alanine aminotransferase	creatine kinase	sodium
alkaline phosphatase	creatinine	total bilirubin
aspartate aminotransferase	glucose	total protein
calcium	inorganic phosphorus	triglycerides
chloride	magnesium	urea
cholesterol total	potassium	protein electrophoresis
cholinesterase		

In M, dose-dependent decreases occurred in AST (26%, 28% and 35% at LD, MD, and HD, respectively) and CK (22%, 48% and 54% at LD, MD and HD, respectively). β-globulin (10-11%) and γ-globulin (26-28%) were decreased in all treatment groups, resulting in a decreased A/G ratio (17-21%). Choline was increased in all treatment groups (25-33%) as was albumin (8-14%).

In F, there were dose-dependent decreases in CK (27%, 33% and 42% at LD, MD, and HD, respectively), β-globulin (5%, 9% and 12% at LD, MD, and HD, respectively) and γ-globulin (10%, 21% and 30% at LD, MD, and HD, respectively). Triglycerides (68%) were increased at the HD.

Gross Pathology

Gross pathology was assessed in all animals.

Small thymuses were observed at the MD in M.

Organ Weights

At necropsy, the following organs were weighed:

adrenals*	lungs	testes*
brain	ovaries*	thymus
heart	pituitary	thyroid with parathyroids
kidneys*	prostate	uterus
liver	spleen	

* Paired organs weighed together

In M, there were increases in relative weights of lungs (12%) and heart (7%) at the LD. At the MD, lungs (24%), heart (10%) and testes (12%) were increased, whereas prostate (10%) was decreased. At the HD, liver (8%), lungs (46%), heart (21%) and brain (7%) were increased, whereas prostate (22%) was decreased. Decreased weights were observed for thymus (8-27%) at all doses and spleen (10%) at the HD.

In F, there were increases in relative weights of heart (5%), adrenals (19%) and ovary (20%) at the LD. At the MD, lungs (5%), heart (8%), adrenals (35%) and ovary (29%) were increased. At the HD, liver (9%), lungs (20%), heart (17%), adrenals (35%), ovary (19%) and brain (6%) were increased, whereas spleen (8%) was decreased.

Histopathology

At necropsy, the following organs, as listed in the study report, were fixed in 10% formalin, except for testes and epididymides, which were fixed in Davidson's solution. Eyes, Harderian, lacrimal and pituitary glands were fixed in 10% formalin within the skull. Lung sections, from selected control and HD animals and all LD and MD animals, were stained by Masson's trichrome method to evaluate smooth muscle and connective tissue. Additional lung sections from selected control and HD animals were stained with Periodic Acid Schiff's reagent for hyaline material.

adrenals	knee joint	seminal vesicles
aorta	lacrimal glands	skeletal muscle
bone marrow (in bone)	liver	skin
brain	lungs	spinal cord
cecum	lymph node: mandibular	spleen
colon	lymph node: bronchial	sternum
duodenum	lymph node: mesenteric	stomach
epididymides	mammary gland	testes
esophagus	ovaries	thymus
eyes with optic nerves	pancreas	thyroid with parathyroids
Harderian glands	peripheral nerves	tongue
heart	pituitary	trachea
ileum	prostate	urinary bladder
jejunum	rectum	uterus
kidneys	salivary glands	vagina

and all other organs/tissues showing macroscopic abnormalities during necropsy and animal identification.

The following tissues from control and HD animals were prepared for histopathology examination.

adrenals	liver	prostate
bone marrow (sternum)	lungs	spleen
epididymides	lymph node: bronchial	stomach
heart	lymph node: mandibular	testes
jejunum	lymph node: mesenteric	thymus
kidneys	ovaries	thyroid with parathyroids

and all other organs/tissues showing macroscopic abnormalities during necropsy.

Tissue examined in LD and MD animals were lungs, lymph nodes, thymus, spleen and prostate.

Adequate Battery: No, a full battery of tissues was not examined for all doses.
Peer Review: Peer review included review of the draft report, histological slides of all target organs and from at least 10% of the remaining animals/organs.

Organs with findings included atrophy of the lymphoid organs, consistent with a pharmacological action of FTY720. The lungs had smooth muscle hypertrophy at the HD, and collagen and increased macrophages at all doses. There was decreased secretion in the prostate at the LD and HD.

Summary of major microscopic findings

		MALE				FEMALE			
Tissue	**Findings**	**0**	**0.1**	**0.5**	**5**	**0**	**0.1**	**0.5**	**5**
Lungs	S. muscle hypertrophy								
	minimal:	**0/10**	**0/10**	**0/10**	**6/10**	**0/10**	**0/10**	**0/10**	**6/10**
	slight:	**0/10**	**0/10**	**0/10**	**4/10**	**0/10**	**0/10**	**0/10**	**4/10**
	Collagenization								
	minimal:	**0/10**	**3/10**	**8/10**	**10/10**	**0/10**	**8/10**	**9/10**	**10/10**
	Increase macrophages								
	minimal:	**2/10**	**6/10**	**8/10**	**7/10**	**1/10**	**3/10**	**3/10**	**5/10**
	slight:	**0/10**	**0/10**	**0/10**	**3/10**	**0/10**	**0/10**	**0/10**	**5/10**
	Granulocyte infiltrate								
	minimal:	**3/10**	**2/10**	**5/10**	**4/10**	**1/10**	**3/10**	**4/10**	**3/10**
	slight:	**0/10**	**1/10**	**0/10**	**3/10**	**0/10**	**1/10**	**0/10**	**7/10**
	Perivascular cuffing								
	minimal:	**4/10**	**3/10**	**0/10**	**1/10**	**3/10**	**4/10**	**1/10**	**4/10**
	Osseous metaplasia								
	minimal:	**2/10**	**0/10**	**1/10**	**1/10**	**0/10**	**0/10**	**0/10**	**2/10**
	Capillary congestion								
	minimal:	**0/10**	**1/10**	**2/10**	**3/10**	**0/10**	**2/10**	**010**	**1/10**
	slight:	**2/10**	**0/10**	**0/10**	**1/10**	**1/10**	**0/10**	**0/10**	**0/10**
Kidneys	Tubular basophilia								
	minimal:	**5/10**	**0/0**	**0/0**	**7/10**	**9/10**	**0/0**	**0/0**	**8/10**
	Necrosis								
	minimal:	**0/10**	**0/0**	**0/0**	**1/10**	**0/10**	**0/0**	**0/0**	**0/10**
Adrenal gland	Necrosis								
	minimal:	**0/10**	**0/2**	**0/2**	**0/10**	**0/10**	**0/0**	**0/0**	**1/10**

Tissue	Findings	MALE 0	MALE 0.1	MALE 0.5	MALE 5	FEMALE 0	FEMALE 0.1	FEMALE 0.5	FEMALE 5
Spleen	White pulp atrophy								
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	0/10	1/10	3/10	1/10	0/10	0/10	0/10
	moderate:	0/10	9/10	9/10	5/10	0/10	9/10	9/10	1/10
	marked:	0/10	1/10	0/10	2/10	0/10	1/10	1/10	9/10
	Vanish marginal								
	minimal:	0/10	4/10	3/10	0/10	0/10	5/10	2/10	0/10
	slight:	0/10	3/10	5/10	0/10	1/10	5/10	5/10	0/10
	moderate:	0/10	1/10	2/10	3/10	0/10	0/10	3/10	1/10
	marked:	0/10	0/10	0/10	7/10	0/10	0/10	0/10	9/10
	Hyperplasia								
	minimal:	0/10	6/10	4/10	0/10	0/10	5/10	5/10	0/10
	Extra. Hematopoiesis								
	minimal:	4/10	4/10	6/10	3/10	1/10	2/10	0/10	1/10
	slight:	6/10	5/10	4/10	7/10	7/10	1/10	6/10	6/10
	moderate:	0/10	1/10	0/10	0/10	2/10	7/10	4/10	3/10
	Red pulp granulocyte								
	minimal:	8/10	8/10	7/10	1/10	10/10	2/10	5/10	2/10
	slight:	2/10	1/10	0/10	9/10	0/10	0/10	0/10	8/10
Thymus	Atrophy of cortex								
	minimal:	0/9	0/10	0/10	0/10	0/9	0/10	0/10	0/10
	slight:	0/9	0/10	0/10	3/10	0/9	0/10	0/10	2/10
	moderate:	0/9	7/10	10/10	6/10	0/9	9/10	10/10	7/10
	marked:	0/9	3/10	0/10	1/10	0/9	1/10	0/10	1/10
	Enlarged medulla								
	minimal:	0/9	0/10	2/10	1/10	0/9	0/10	0/10	1/10
	slight:	0/9	0/10	2/10	6/10	0/9	1/10	3/10	710
	moderate:	0/9	10/10	6/10	3/10	0/9	9/10	7/10	0/10
	marked:	0/9	0/10	0/10	0/10	0/9	0/10	0/10	0/10
Bronchial Lymph Node	Atrophy								
	minimal:	0/6	1/2	0/1	1/6	0/8	1/5	0/5	0/7
	slight:	0/6	1/2	0/1	4/6	0/8	0/5	4/5	3/7
	moderate:	0/6	0/2	0/1	1/6	0/8	0/5	0/5	4/7
	Granulocyte infiltrate								
	minimal:	0/6	0/2	0/1	0/6	0/8	1/5	4/5	3/7
	slight:	0/6	0/2	0/1	0/6	0/8	0/5	0/5	1/7
Mesenteric Lymph Node	Germinal centers								
	minimal:	7/10	5/10	0/9	0/10	4/10	1/10	0/10	0/9
	slight:	1/10	0/10	0/9	0/10	0/10	0/10	0/10	0/9
	Atrophy								
	minimal:	0/10	3/10	2/9	0/10	0/10	0/10	0/10	0/9
	slight:	0/10	5/10	3/9	2/10	0/10	4/10	1/10	0/9
	moderate:	0/10	1/10	4/9	6/10	1/10	6/10	9/10	2/9
	marked:	0/10	0/10	0/9	2/10	0/10	0/10	0/10	7/9
	Sinus histiocytosis								
	minimal:	4/10	6/10	3/9	1/10	4/10	0/10	1/10	2/9
	slight:	1/10	4/10	6/9	6/10	2/10	5/10	7/10	4/9
	moderate:	0/10	0/10	0/9	3/10	0/10	5/10	2/10	3/9
	Hyperplasia								
	minimal:	1/10	5/10	3/9	5/10	0/10	0/10	4/10	4/9
	slight:	0/10	1/10	1/9	5/10	0/10	7/10	0/10	1/9
	Granulocyte infiltrate								
	minimal:	0/10	0/10	0/9	1/10	0/10	1/10	3/10	2/9

Tissue	Findings	MALE 0	MALE 0.1	MALE 0.5	MALE 5	FEMALE 0	FEMALE 0.1	FEMALE 0.5	FEMALE 5
Mesenteric	Vasculopathy								
Lymph	minimal:	**0/10**	**1/10**	**0/9**	**1/10**	**0/10**	**0/10**	**0/10**	**1/9**
Node	slight:	**0/10**	**0/10**	**0/9**	**1/10**	**0/10**	**0/10**	**0/10**	**0/9**
Mandibular	Germinal centers								
Lymph	minimal:	**0/8**	**3/7**	**5/6**	**3/7**	**3/10**	**2/7**	**1/7**	**1/10**
Node	slight:	**6/8**	**0/7**	**0/6**	**0/7**	**6/10**	**4/7**	**1/7**	**2/10**
	moderate:	**1/8**	**1/7**	**0/6**	**0/7**	**1/10**	**1/7**	**1/7**	**0/10**
	Atrophy								
	minimal:	**0/8**	**5/7**	**1/6**	**1/7**	**0/10**	**0/7**	**3/7**	**1/10**
	Slight:	**0/8**	**0/7**	**1/6**	**3/7**	**0/10**	**0/7**	**2/7**	**4/10**
	moderate:	**0/8**	**0/7**	**0/6**	**2/7**	**0/10**	**0/7**	**0/7**	**4/10**
	Hyperplasia								
	minimal:	**5/8**	**6/7**	**5/6**	**5/7**	**8/10**	**4/7**	**6/7**	**4/10**
	slight:	**1/8**	**0/7**	**0/6**	**0/7**	**1/10**	**3/7**	**1/7**	**0/10**
	Granulocyte infiltrate								
	minimal:	**0/8**	**0/7**	**1/6**	**1/7**	**1/10**	**2/7**	**3/7**	**2/10**
	Slight:	**0/8**	**0/7**	**0/6**	**2/7**	**0/10**	**0/7**	**0/7**	**1/10**
Prostate	Secretion decrease								
	minimal:	**0/9**	**0/10**	**2/10**	**7/10**	**-**	**-**	**-**	**-**
	Inspissated contents								
	minimal:	**0/9**	**1/10**	**1/10**	**4/10**	**-**	**-**	**-**	**-**
	Inflammation								
	minimal:	**0/9**	**0/10**	**0/10**	**1/10**	**-**	**-**	**-**	**-**

Extra. - extramedullary

Toxicokinetics

Blood samples were taken from all groups in Weeks 4 and 13 at 1, 2, 4, 7 and 24 hours post-dose.

Plasma C_{max} and AUC increased approximately dose-proportionally in M and F, with generally no sex differences. Accumulation of FTY720 was observed at Week 13 with increases of 1.51-1.78-fold. T_{max} was observed between 1-7 hours.

Table of toxicokinetic parameters (Sponsor's)

Period	week 4			week 13		
Dose [mg/kg/day]	0.1	0.5	5	0.1	0.5	5
Males						
t_{max} [h]	7	1	2	4	7	1
C_{max} [ng/mL]	6	22	207	8	33	333
C_{max}/dose(1)	58	43	41	80	66	67
AUC (0-24h) [h·ng/mL]	105	410	3858	164	659	6015
AUC (0-24h)/dose(2)	1048	821	772	1637	1319	1203
Females						
t_{max} [h]	2	2	2	2	7	7
C_{max} [ng/mL]	7	22	210	9	36	335
C_{max}/dose(1)	68	44	42	90	72	67
AUC (0-24h) [h·ng/mL]	115	429	4264	174	763	6983
AUC (0-24h)/dose(2)	1153	858	853	1744	1525	1397

(1): [(ng/mL)/(mg/kg/day)]
(2): [(h·ng/mL)/(mg/kg/day)]

Stability and Homogeneity

Homogeneity testing demonstrated that solutions were homogeneously distributed (1.9-13.1% at three different levels). FTY720 was chemically stable for up to 6 hours at room temperature. Samples were taken from target solutions of 0.02, 0.1 and 1.0 mg/ml at different time points that included; initial, 4 and 6 hours at room temperature. Stability data showed that the solutions ranged between 97.9-100.7% of the target concentration.

Study title: 6-Month oral toxicity study in rats

Study no.: PCS-r7219

Study report location: EDR: 4.2.3.2.1

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: 25 August 1994

GLP compliance: Yes [redacted] (b) (4), 1982, 1988)

QA statement: Yes (Dated: 7 Mar 1996, amended 28 Aug 1998)

Drug, lot #, and % purity: Y-32720, Lot M, 100.3%

Key Study Findings

- A male administered 0.01 mg/kg/day and one at 10 mg/kg/day died with pulmonary edema and hydrothorax and a second 10 mg/kg/day male died with foamy contents in the trachea.
- Lymphoid organ atrophy was observed at all doses, smooth muscle hypertrophy in the lungs at the 10 mg/kg/day, and lung and heart weights were increased at 10 mg/kg/day.

- Excluding lymphoid atrophy and the 1 male that died at 0.01 mg/kg/day, the NOAEL was 0.3 mg/kg/day.

Methods

Doses:	0, 0.01, 0.03, 0.3 and 10 mg/kg/day (C, LD, MLD, MHD and HD)
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	4 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Rat/SD
Number/Sex/Group	
Main study:	12
Toxicokinetic study:	4 for Day 1, 4 for Week 13 (HD only)
Age:	5 weeks at start of treatment
Weight	
Males:	114.0-134.7 g
Females:	114.3-129.4 g

Observations and Results

Mortality

One control M with broken teeth was sacrificed early in Week 26 due to reduced body weight. One M at the LD died in week 24 and 2 at the HD died in Week 16 and 25 with normal body weights, food consumption and no clinical signs. The M at the LD had pulmonary edema and hydrothorax and 1 M at the HD died with a dark red lung and hydrothorax with the second HD dying with foamy contents in the trachea. The Sponsor considered these deaths to be respiratory failure due to misdosing

Clinical Signs

Observations were performed daily pre-treatment and more than once post-dose.

Salivation occurred for HD animals; otherwise, there were no treatment-related signs.

Body Weights

Body weights were recorded pre-treatment, twice weekly during treatment until Week 4 and then once weekly until the end of treatment.

A decrease in body weight gain was observed at the HD from Day 3 on; total weight gain was decreased by 21% and 15% for HD M and F, respectively.

Food Consumption

Food consumption was determined for pairs of animals pre-treatment and weekly during treatment.

A decrease in food consumption was observed in HD animals from Week 1 on.

Ophthalmoscopy
Examinations of all rats were performed in Weeks 13 and 26.

No ophthalmoscopy data were submitted. Findings reported included a fundus hemorrhage in the left eye of a F at the MHD.

Hematology
Blood samples were taken from all animals at the end of the study. The following parameters were assessed: RBC, WBC and platelet counts, HGB and HCT, MCV, MCH, MCHC, reticulocytes, PT and APTT.

At the HD, there was an increase in RBC parameters; HGB, HCT and MCV. Reticulocytes were increase in M at all doses and at the HD in F. WBC counts were decreased at all doses, with lymphocytes decreased and neutrophils increased.

Clinical Chemistry
Blood samples were taken from all animals at the end of the study. The following parameters were assessed: GOT, GPT, ALP, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

In M, there were increases in GOT (41%, 57%, 49% and 103%), phosphorus (13%, 11%, 17% and 24%) and α_2-globulin (11%, 8%, 10% and 11%) and decrease in γ-globulin (5%, 24%, 31% and 34%) at the LD, MLD, MHD and HD, respectively. At the HD, there were also increases in GPT (133%), total bilirubin (117%), phospholipids (26%) and decrease in total protein (5%).

In F, there were increases in GOT (2%, 56%, 44% and 48%), GPT (33%, 103%, 47% and 86%), total cholesterol (10%, 30%, 35% and 26%), phospholipids (9%, 22%, 26% and 22%) and decrease in γ-globulin (8%, 39%, 47% and 44%) at the LD, MLD, MHD and HD, respectively. At the HD, there were also increases in total bilirubin (89%), triglycerides (145%) and decrease in total protein (7%).

Urinalysis
Urine samples were taken from all animals at Weeks 13 and 26.

There appeared no related treatment-related changes in urinalysis at Week 13. In Week 26 there were changes in F at the HD that included statistically significant increases specific gravity, sodium, potassium and chloride.

Gross Pathology
Gross pathology was performed at necropsy after last administration or as soon as possible after early death.

Spleen size was decreased in 1, 1, 2 and 4 M at the LD, MLD, MHD and HD, respectively and 1 and 2 F at the MHD and HD, respectively. There was hepatization in the upper lobe of 1 M at the HD.

Organ Weights

At necropsy the following organs were weighed: brain, heart, lungs, liver, kidneys, spleen, submandibular glands and testes. The following organs were fixed in 10% formalin and weighed: pituitary, adrenals, thymus, thyroid glands, seminal vesicles, prostate, ovary and uterus.

In M, relative weight of spleen was decreased at the MHD (14%) and HD (21%). At the HD, relative weight of lung (39% and 32%), heart (12% and 23%) and brain (14% and 10%) were increased in M and F, respectively and testes (21%) and uterus (43%).

Histopathology

At necropsy the following organs were prepared for histopathology examination: liver, kidneys, lungs, heart, spleen, pancreas, brain, spinal cord, adrenals, thyroid glands, pituitary, thymus, submandibular lymph node, mesenchymal lymph node, femur bone, parotid glands, submandibular glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, bladder, aorta, seminal vesicles, prostate, ovary, uterus, vagina, mammary gland, skin, skeletal muscle, tongue and sternum. Tissues were fixed in 10% formalin, except for testes, epididymis, Harderian glands and eye balls, which were fixed in Bouin's solution.

Adequate Battery: Yes
Peer Review: No

Organs with findings included atrophy of the lymphoid organs consistent with a pharmacological action of FTY720. Lungs had muscle hypertrophy at the HD. Other findings included a glioma in 1 F at the LD and testes degeneration and epididymis vacuolation in 1 M at the HD. There were 3 animals that died; 1 and 2 M at the LD and HD, respectively, all had pulmonary edema, with macrophage infiltration observed in 1 at the LD and 1 at the HD.

Summary of major microscopic findings

		MALE					FEMALE				
Tissue	**Findings**	**0**	**0.01**	**0.03**	**0.3**	**10**	**0**	**0.01**	**0.03**	**0.3**	**10**
Brain	Glioma										
	very slight:	**0/11**	**0/11**	**0/12**	**0/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**0/12**
	slight:	**0/11**	**0/11**	**0/12**	**0/12**	**0/10**	**0/12**	**1/12**	**0/12**	**0/12**	**0/12**
Pituitary	Foamy cells										
	very slight:	**0/11**	**0/11**	**1/12**	**0/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**0/12**
	Cyst										
	very slight:	**0/11**	**0/11**	**0/12**	**0/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**0/12**
	slight:	**0/11**	**0/11**	**0/12**	**1/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**1/12**
	Altered cell foci										
	very slight:	**0/11**	**0/11**	**0/12**	**0/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**0/12**
	slight:	**0/11**	**0/11**	**0/12**	**1/12**	**0/10**	**0/12**	**0/12**	**0/12**	**0/12**	**0/12**

		MALE					FEMALE				
Tissue	**Findings**	**0**	**0.01**	**0.03**	**0.3**	**10**	**0**	**0.01**	**0.03**	**0.3**	**10**
Lungs	Cell infiltration										
	very slight:	0/11	0/11	0/12	0/12	1/10	0/12	0/12	0/12	0/12	0/12
	Muscle hypertrophy										
	very slight:	0/11	0/11	0/12	0/12	7/10	0/12	0/12	0/12	0/12	9/12
Liver	Hepatocyte necrosis										
	very slight:	0/11	0/11	0/12	0/12	0/10	1/12	1/12	0/12	1/12	2/12
	slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	0/12	0/12	2/12
Spleen	Hematopoiesis										
	very slight:	0/11	0/11	0/12	0/12	1/10	0/12	0/12	0/12	0/12	3/12
	slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	0/12	0/12	1/12
	Vesicle capsule										
	very slight:	0/11	0/11	0/12	1/12	0/10	0/12	0/12	0/12	0/12	0/12
	slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	0/12	0/12	0/12
	White pulp atrophy										
	very slight:	0/11	10/11	4/12	4/12	1/10	0/12	9/12	0/12	1/12	0/12
	slight:	0/11	0/11	8/12	4/12	5/10	0/12	3/12	7/12	8/12	10/12
	moderate:	0/11	0/11	0/12	4/12	4/10	0/12	0/12	5/12	3/12	2/12
Thymus	Atrophy of cortex										
	very slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	0/12	0/12	0/12
	slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	0/12	0/12	2/12
	moderate:	0/11	0/11	0/12	1/12	0/10	0/12	0/12	0/12	4/12	2/12
	severe:	0/11	0/11	0/12	11/12	10/10	0/12	0/12	0/12	8/12	8/12
	Enlarged medulla										
	very slight:	0/11	8/11	1/12	0/12	0/10	0/12	3/12	0/12	0/12	0/12
	slight:	0/11	0/11	8/12	1/12	0/10	0/12	9/12	6/12	1/12	2/12
	moderate:	0/11	0/11	3/12	11/12	10/10	0/12	0/12	6/12	11/12	10/12
Mesenteric Lymph Node	Atrophy										
	very slight:	0/11	3/11	4/12	4/12	1/10	0/12	5/12	4/12	1/12	0/12
	slight:	0/11	0/11	0/12	4/12	4/10	0/12	6/12	6/12	9/12	5/12
	moderate:	0/11	0/11	0/12	1/12	4/10	0/12	0/12	2/12	2/12	7/12
	severe:	0/11	0/11	0/12	0/12	1/10	0/12	0/12	0/12	0/12	0/12
Submandibular Lymph Node	Atrophy										
	very slight:	0/11	1/11	1/12	1/12	1/10	0/12	1/12	9/12	5/12	4/12
	slight:	0/11	0/11	0/12	0/12	0/10	0/12	0/12	2/12	1/12	7/12
Testes	Degeneration										
	very slight:	0/11	0/11	0/12	0/12	0/10	-	-	-	-	-
	slight:	0/11	0/11	0/12	0/12	0/10	-	-	-	-	-
	moderate:	0/11	0/11	0/12	0/12	0/10	-	-	-	-	-
	severe:	0/11	0/11	0/12	0/12	1/10	-	-	-	-	-
Epididymides	Vacuolation										
	very slight:	0/11	0/11	0/12	0/12	0/10	-	-	-	-	-
	slight:	0/11	0/11	0/12	0/12	1/10	-	-	-	-	-

Toxicokinetics (Study №: DMPK-r7070)

Blood samples were taken from 4 M and 4 F at the HD on Day 1 and in Week 26 at 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 hours post-dose. There were 2 animals per time point.

T_{max} was 8 and 9 hours for M and F, respectively, in Week 0 and 9.3 and 4.5 hours in Week 26. C_{max}'s were 225.0 and 453.2 ng/ml for M in Week 0 and 26 and 223.2 and 532.6 ng/ml for F in Week 0 and 26, respectively an approximate 2-fold accumulation.

Table of toxicokinetic parameters (Sponsor's)

Dose (mg/kg)	Sex	Week		0.5hr	1hr	2hr	4hr	8hr	12hr	24hr	36hr	48hr	72hr
10	Male	0	N	4	4	4	4	4	4	3[a]	4	4	4
			Mean	25.5	42.9	79.0	144.2	225.0	178.8	125.9	107.4	85.3	31.6
			S.D.	9.0	9.4	33.3	31.3	7.7	10.3	12.6	12.8	18.5	7.4
		13	N					4		4			
			Mean					552.0		333.2			
			S.D.					71.5		28.7			
		26	N	3[b]	3	3	3	3	3	3	3	3	3
			Mean	311.0	347.8	304.3	341.2	376.5	443.5	258.9	273.6	196.0	152.7
			S.D.	29.8	53.3	22.5	67.9	48.9	82.8	74.4	97.1	48.6	61.6
	Female	0	N	4	4	4	4	4	4	4	4	4	4
			Mean	24.2	44.0	76.9	143.9	220.4	212.4	129.7	115.9	59.3	44.8
			S.D.	12.0	11.4	21.5	9.9	11.8	5.7	9.1	15.9	5.9	12.8
		13	N					4		4			
			Mean					677.3		348.8			
			S.D.					197.7		77.4			
		26	N	4	4	4	4	4	4	4	4	4	4
			Mean	391.5	424.7	515.3	381.3	376.7	390.1	298.5	349.3	253.3	174.7
			S.D.	86.6	76.6	204.3	72.7	55.0	51.7	43.9	112.5	83.2	74.4

a) Not assayed (No. 7101)
b) One rat was dead (No. 6103)

(unit : ng/ml)

Study title: 26-Week oral toxicity study in rats

Study no.:	PCS-r991052
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	Novartis Pharma AG, Basel, Switzerland.
Date of study initiation:	28 June 1999
GLP compliance:	Yes (Switzerland, 1986 and 2000)
QA statement:	Yes (Dated: 4 Apr 2001)
Drug, lot #, and % purity:	Y-32720, Lot № 9922006, 100%

Key Study Findings

- Several animals died or were killed before scheduled termination. Renal and vascular lesions resulting from treatment caused one 7.5 mg/kg/day male death and foamy outflow of the lungs was observed in one 1.5 and two 7.5 mg/kg/day animals.
- Lungs, heart and liver were increased in weight at all doses.
- Lymphoid organs showed atrophy at all doses. Other findings included lung smooth muscle hypertrophy and osseous metaplasia and cataracts. At 7.5 mg/kg/day, several organs had artery wall thickening, heart

degeneration/necrosis and wall thickening in males and females, and microvacuolation in the cerebrum was detected in males.
- Due to smooth muscle hypertrophy in the lungs, a NOAEL was not established.

Methods

Doses:	0, 0.3, 1.5 and 7.5 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	(b) (4) water
Species/Strain:	Rat/Wist Han
Number/Sex/Group	
Main study:	20
Recovery:	10/sex for Control, MD and HD groups
Age:	8 weeks at start of treatment
Weight	124-259 g
Deviation from study protocol:	Two animals (№s. 144 (MD F) and 196 (HD F)) were replaced on Day 7 of treatment due to a tail lesion or because of a dosing error. Toxicokinetic blood samples taken from control animals were not analyzed for the metabolites M2 and M3 due to insufficient sample volume.

Observations and Results

Mortality

Observations were performed at least daily during pre-treatment and recovery and twice daily during treatment.

One M and 1 F at the LD, 2 F at the MD and 4 M and 2 F at the HD died or were killed during treatment on Days 169, 149, 53 and 92, 109, 118, 145 and 197 and 49 and 109, respectively. Renal and vascular lesions resulting from drug treatment caused 1 M death at the HD. Two animals, 1 MD and 1 HD, were accidently killed with the cause of the other deaths unknown, although foamy outflow of the lungs was observed in 1 MD and 2 HD of these animals.

Clinical Signs

Observations were performed at least daily during pre-treatment and recovery and twice daily during treatment.

Dyspnea, hypoactivity, abnormal gait and hunched posture were observed in 1 M at the LD from Week 17, and 1 F at the MD displayed labored breathing on one occasion. Otherwise, there was no drug-related change in clinical signs.

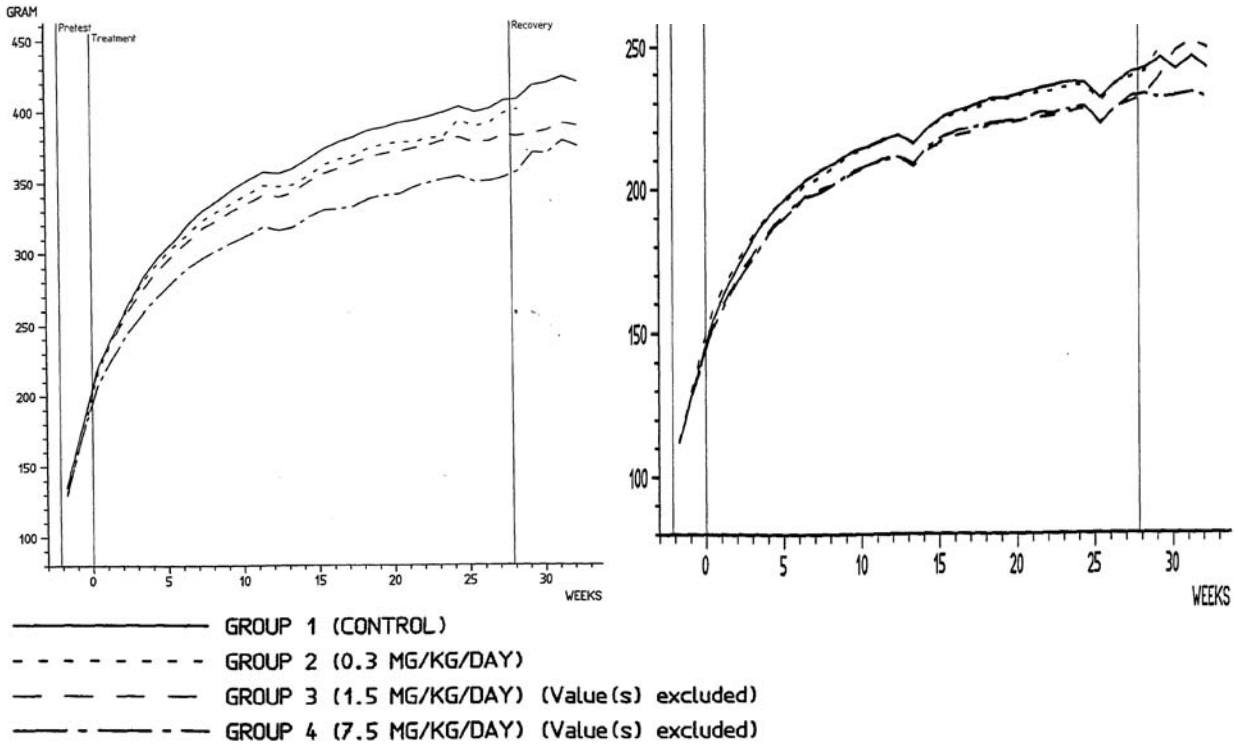
Body Weights

Body weights were recorded once weekly pre-treatment and during recovery and daily during treatment.

M and F at the MD and greater, a decrease in body weight gain was observed, with 10% and 20% decreases at MD and HD in M and a 7% decrease in F at MD and HD.

Graphs of body weights (Sponsor's)

MALE **FEMALE**



Food Consumption

Food consumption was determined for cages once weekly from pre-treatment until the end of recovery.

There was a decrease in food consumption in Week 1 observed in animals at the HD. Food consumption returned to normal during the recovery period.

Ophthalmoscopy

Examination of all rats was performed in Weeks 13 and 25.

One F at the HD had bleeding of the left eye vitreous body at Weeks 13 and 25; otherwise there were no drug-related ophthalmological findings.

Hematology

Blood samples were taken in Weeks 13/14 and 26. The following parameters, listed in the study report, were assessed.

erythrocytes	mean corpuscular hemoglobin	reticulocytes
fibrinogen***	mean corpuscular hemoglobin concentration	thrombin time***
hematocrit	mean corpuscular volume	thrombocytes
hemoglobin	prothrombin time***	white blood cell count**
inclusion bodies*	activated partial thromboplastin time***	red cell distribution width

* Counted when abnormalities were present

** Total and differential

*** Coagulation parameters.

In Week 13, M had a dose-dependent increase in reticulocytes of 6%, 8% and 17% at LD, MD and HD, respectively. WBCs, lymphocytes and monocytes were decreased in all dose groups; neutrophils were increased. In Week 26, reticulocytes in M were still increased by 6%, 14% and 14% at LD, MD and HD, respectively, and WBCs, lymphocytes and monocytes were decreased, while neutrophils were increased. Coagulation times were decreased at Weeks 13 and 26.

In Week 13, F had an increase in RBCs and a dose-dependent increase in reticulocytes of 7%, 13% and 25% at LD, MD and HD, respectively. WBCs, lymphocytes and monocytes were decreased in all dose groups; neutrophils were increased. In Week 26, reticulocytes in F were still increased by 8%, 16% and 26% at LD, MD and HD, respectively and WBCs and lymphocytes were decreased, while neutrophils were increased. Coagulation times were decreased at Weeks 13 and 26.

Reversal of reticulocyte and neutrophil increases was observed, but no reversal of WBCs, lymphocyte or monocytes decrease.

Clinical Chemistry

Blood samples were taken in Weeks 13/14 and 26. The following parameters, listed in the study report, were assessed.

alanine aminotransferase	creatine kinase	sodium
alkaline phosphatase	creatinine	total bilirubin
aspartate aminotransferase	glucose	total protein
calcium	inorganic phosphorus	triglycerides
chloride	magnesium	urea
cholesterol total	potassium	
cholinesterase	protein electrophoresis	

No anticoagulant was used.

In M at the LD in Week 13/14, increases occurred in urea (2%), glucose (18%) and triglycerides (37%) and decreases occurred in CK (35%). At the MD, increases occurred in urea (11%), glucose (20%) and triglycerides (24%) and decreases occurred in AST (18%) and CK (58%). At the HD, increases occurred in urea (19%), glucose (14%) and triglycerides (64%) and decreases occurred in AST (33%) and CK (68%). The globulins, α_2-globulin (5-8%), β-globulin (6-10%) and γ-globulin (20-32%) were decreased in all treatment groups and resulted in a decreased A/G ratio. Changes at the HD also included decreases in potassium and magnesium and an increase in calcium ions. At Week 26, CK and globulins were still decreased; urea and triglycerides were still increased. Increases in creatinine (4%, 9% and 12%; LD, MD and HD, respectively) and choline (~20%) were observed in all treatment groups.

In F, dose-dependent decreases were observed at the LD, MD and HD in ALT (16%, 20% and 22%), AST (10%, 18% and 21%) and CK (21%, 48% and 47%). Globulins were decreased in all treatment groups; α_2-globulin (8-14%), β-globulin (11-15%) and γ-globulin (36-40%). Increases occurred in urea (11-26%) and triglycerides (17-52%). A decrease in magnesium and an increase in phosphorus were observed at the HD. In F at Week 26, there was still a slight decrease in CK and globulins, with urea, triglycerides and phosphorus still increased.

Slight recovery was observed in CK, urea and triglyceride in males, but globulins were still decreased and potassium and magnesium were still increased. Phosphorus had decreased (20%). Similar findings were seen in F after recovery, although creatinine had recovered.

Urinalysis

Urine samples were taken from all animals at Weeks 13/14 and 26. The following parameters, listed in the study report, were assessed.

bilirubin*	nitrite*	specific gravity
color/clarity	occult blood*	urobilinogen*
glucose*	pH*	volume
ketone bodies*	protein*	
leukocytes*	sediment (microscopically)**	

* Test strips

** The last 10 surviving animals from each sex/group were examined

At the HD, in Week 13 there was an increase in urine volume, specific gravity and protein. These changes were not observed in M at the HD in Week 26, but were still evident in F at the HD. Increased specific gravity was still evident at the HD in M and F at the end of the recovery period

Gross Pathology
Gross pathology was assessed in all animals.

Irregular surface of the kidney was observed in 1 M at the HD that died early. Other macroscopic findings in animals that died early included foamy bronchial outflow from the lungs in 1 F at the LD, 1 M at the HD and 1 F at the HD.

Organ Weights
At necropsy, the following organs, listed in the study report, were weighed.

adrenals*	liver	spleen
brain	lungs	testes*
heart	ovaries*	thymus
kidneys*	pituitary	thyroid with parathyroids
	prostate	uterus

*paired organs weighed together.

In M, relative to body weight, there was a dose-dependent increase in mean lung (10%, 18% and 38%), heart (6%, 14% and 19%), liver (5%, 6% and 12%), testes (4%, 9% and 13%) and adrenal gland (12%, 15% and 17%) weights at the LD, MD and HD, respectively. Thymus, spleen and prostate weights were decreased at all doses, with the brain was increased in M at the HD. Absolute kidney weights were decreased by 2%, 4% and 11% at the LD, MD and HD, respectively.

A dose-dependent increase in F mean lung (17%, 27% and 40%), heart (2%, 12% and 25%), liver (4%, 2% and 8%), ovary (5%, 9% and 19%) and adrenal gland (3% and 13%) weights at the LD, MD and HD, respectively was observed. The thymus was decreased at all doses; the brain increased in F at the HD. Absolute kidney weights were decreased by 6%, 11% and 10% at the LD, MD and HD, respectively.

In M, there was partial recovery in heart (6% and 13%), lung (6% and 9%), adrenal glands (13% and 9%), testes (4% and 10%) and brain (10% and 15%) weight at the MD and HD, the thymus was still decreased. In F, recovery was partial for heart (8% and 9%), lungs (3% and 8%) and adrenal glands (24% and 21%) at the MD and HD.

Histopathology
At necropsy, the following organs, listed in the study report, were prepared for histopathology examination. Tissues were fixed in 10% formalin, except for testes, epididymides, prostate, seminal vesicles, eyes, Harderian glands and lacrimal glands that were fixed in Davidson's solution.

adrenals	knee joint	seminal vesicles
aorta	lacrimal glands	skeletal muscle
bone marrow (in bone)	liver	skin
brain	lungs	spinal cord
cecum	lymph node: mandibular	spleen
colon	lymph node: bronchial	sternum
duodenum	lymph node: mesenteric	stomach
epididymides	mammary area	testes
esophagus	ovaries	thymus
eyes with optic nerves	pancreas	thyroid with parathyroids
Harderian glands	peripheral nerves	tongue
heart	pituitary	trachea
ileum	prostate	urinary bladder
jejunum	rectum	uterus
kidneys	salivary glands	vagina

and all other organs/tissues which showed macroscopic abnormalities during necropsy and the animal identification (implanted microchip).

Adequate Battery: Yes

Peer Review: Results of the post-mortem examination were reviewed by another pathologist. Peer review consisted of a review of the draft pathology report, the histological slides from all target organs and at least 10% of the animals/organs and additional raw data (necropsy and organ weight tables).

There was lymphoid organ atrophy in all treatment groups consistent with a pharmacological action of FTY720. Other prominent findings included lung smooth muscle hypertrophy and osseous metaplasia, kidney basophilic tubules and increased hyaline casts, in all treated groups. At the HD, artery wall thickening was observed in several organs and cataracts were observed in 2 M and 1 F at the HD. Heart degeneration/necrosis and wall thickening was observed at the HD in M, the prostate had decreased secretory production in all treated groups and 1 M at the HD had microvacuolation in the cerebrum. The heart findings reversed after the recovery period. Slight recovery of lung smooth muscle hypertrophy and kidney basophilic tubules and the lymphoid organ atrophy was observed.

Summary of major male microscopic findings

Tissue	Findings	Main Study 0	Main Study 0.3	Main Study 1.5	Main Study 7.5	Recovery 0	Recovery 1.5	Recovery 7.5
Brain	Mineralization							
	minimal:	**0/20**	**0/1**	**0/0**	**1/21**			
	Microvacuolation							
	minimal:	**0/20**	**0/1**	**0/1**	**1/21**			
Eyes	Inflammation							
	minimal:	**0/20**	**1/1**	**0/0**	**0/21**			
	Conjunctivitis							
	minimal:	**0/20**	**0/1**	**0/0**	**1/21**			
	Cataracts							
	minimal:	**0/20**	**0/1**	**0/0**	**1/21**			
	slight:	**0/20**	**0/1**	**0/0**	**1/21**			
Mammary area	Fibrinoid necrosis							
	minimal:	**0/18**	**0/0**	**0/19**	**1/18**			
	Artery wall thickening							
	minimal:	**0/18**	**0/0**	**0/19**	**1/18**			
Heart	Myocardial degeneration							
	minimal:	**0/20**	**1/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/20**	**3/21**	**0/10**	**0/0**	**0/9**
	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/20**	**2/21**	**0/10**	**0/0**	**0/9**
	Mono. cell infiltration							
	minimal:	**0/20**	**0/1**	**0/20**	**1/21**	**2/10**	**0/0**	**0/9**
	Epicarditis							
	minimal:	**0/20**	**0/1**	**0/20**	**0/21**	**0/10**	**0/0**	**1/9**
Lungs	Lymphoid cuffing							
	minimal:	**6/20**	**0/20**	**2/20**	**5/20**	**2/10**	**6/10**	**3/9**
	slight:	**1/20**	**0/20**	**0/20**	**0/20**	**2/10**	**3/10**	**0/9**
	Inflammatory focus							
	minimal:	**3/20**	**0/20**	**0/20**	**2/20**	**0/10**	**2/20**	**0/9**
	Smooth muscle hypertrophy							
	minimal:	**0/20**	**2/20**	**9/20**	**18/20**	**0/10**	**3/10**	**4/9**
	slight:	**0/20**	**0/20**	**1/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Osseous metaplasia							
	minimal:	**0/20**	**1/20**	**1/20**	**2/20**	**0/10**	**0/10**	**1/9**
	Hemorrhage							
	minimal:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	moderate:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	Edema							
	minimal:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	moderate:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	Alveolar macrophage							
	minimal:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	Congestion							
	present:	**0/20**	**0/20**	**0/20**	**4/20**	**0/10**	**0/10**	**0/9**
Liver	Vacuolation							
	minimal:	**7/20**	**0/1**	**9/20**	**8/21**	**8/10**	**0/0**	**4/9**
	slight:	**7/20**	**0/1**	**2/20**	**3/21**	**0/10**	**0/0**	**1/9**
	Inflammatory cell infiltration							
	minimal:	**6/20**	**0/1**	**10/20**	**9/21**	**2/10**	**0/0**	**1/9**

Tissue	Findings	Main Study 0	0.3	1.5	7.5	Recovery 0	1.5	7.5
Liver	Bile duct hyperplasia							
	minimal:	**0/20**	**0/1**	**1/20**	**0/21**	**0/10**	**0/0**	**0/9**
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**
Kidneys	Basophilic tubules							
	minimal	**5/20**	**10/20**	**15/20**	**9/20**	**4/10**	**9/10**	**8/9**
	slight:	**0/20**	**0/20**	**3/20**	**5/20**	**0/10**	**0/10**	**0/9**
	moderate:	**0/20**	**0/20**	**1/20**	**2/20**	**0/10**	**0/10**	**0/9**
	marked:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Tubular hypertrophy							
	minimal:	**0/20**	**1/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	Tubular hyperplasia							
	minimal:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**1/9**
	Hyaline cast							
	minimal	**1/20**	**1/20**	**2/20**	**6/20**	**1/10**	**1/10**	**2/9**
	slight:	**0/20**	**0/20**	**1/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Hyaline droplets							
	minimal:	**0/20**	**1/20**	**0/20**	**2/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**1/20**	**0/20**	**0/10**	**0/10**	**0/9**
	Artery wall thickening							
	minimal:	**0/20**	**0/20**	**1/20**	**1/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**1/20**	**2/20**	**0/10**	**0/10**	**0/9**
	Fibrinoid necrosis							
	minimal:	**0/20**	**0/20**	**1/20**	**2/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	moderate:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Hemorrhage							
	minimal:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Cyst							
	minimal:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**1/20**	**0/10**	**0/10**	**0/9**
	Mineralization							
	minimal:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	slight:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	moderate:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
	severe:	**0/20**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**0/9**
Thyroid glands	Artery wall thickening							
	minimal:	**0/19**	**0/0**	**0/20**	**1/21**			
	Fibrinoid necrosis							
	minimal:	**0/19**	**0/0**	**0/20**	**1/21**			
Adrenal glands	Vacuolation							
	minimal:	**1/20**	**0/1**	**0/0**	**3/21**			
	Hemorrhage/necrosis							
	minimal:	**0/20**	**0/1**	**0/0**	**0/21**			
	slight:	**0/20**	**1/1**	**0/0**	**0/21**			
Spleen	Increased hematopoiesis							
	minimal:	**1/19**	**1/20**	**2/20**	**1/21**	**4/10**	**2/9**	**2/9**
	slight:	**0/19**	**0/20**	**1/20**	**3/21**	**3/10**	**6/9**	**4/9**
	moderate	**0/19**	**0/20**	**0/20**	**0/21**	**3/10**	**1/9**	**1/9**
	severe:	**0/19**	**0/20**	**0/20**	**0/21**	**0/10**	**0/9**	**0/9**

Tissue	Findings	Main Study 0	Main Study 0.3	Main Study 1.5	Main Study 7.5	Recovery 0	Recovery 1.5	Recovery 7.5
Spleen	Lymphoid atrophy							
	minimal:	**0/19**	**17/20**	**12/20**	**12/21**	**1/10**	**0/9**	**4/9**
	slight:	**0/19**	**2/20**	**6/20**	**8/21**	**0/10**	**0/9**	**0/9**
	Artery wall thickening							
	minimal:	**0/19**	**0/20**	**1/20**	**1/21**	**0/10**	**0/9**	**0/9**
	slight:	**0/19**	**0/20**	**0/20**	**3/21**	**0/10**	**0/9**	**0/9**
	Fibrinoid necrosis							
	minimal:	**0/19**	**0/20**	**0/20**	**1/21**	**0/10**	**0/9**	**0/9**
	slight:	**0/19**	**0/20**	**0/20**	**2/21**	**0/10**	**0/9**	**0/9**
	Hemangiosarcoma							
	present:	**0/19**	**0/20**	**0/20**	**0/21**	**0/10**	**1/9**	**0/9**
Thymus	Atrophy of cortex							
	minimal:	**0/19**	**1/20**	**0/20**	**0/21**	**0/10**	**0/10**	**0/9**
	slight:	**0/19**	**0/20**	**1/20**	**0/21**	**0/10**	**0/10**	**1/9**
	moderate:	**0/19**	**11/20**	**11/20**	**13/21**	**0/10**	**0/10**	**8/9**
	severe:	**0/19**	**8/20**	**8/20**	**8/21**	**0/10**	**0/10**	**0/9**
	Enlarged medulla:							
	minimal:	**0/19**	**1/20**	**0/20**	**0/21**	**0/10**	**0/10**	**0/9**
	slight:	**0/19**	**0/20**	**1/20**	**0/21**	**0/10**	**0/10**	**0/9**
	moderate:	**0/19**	**11/20**	**11/20**	**13/21**	**0/10**	**0/10**	**9/9**
	severe:	**0/19**	**8/20**	**8/20**	**8/21**	**0/10**	**0/10**	**0/9**
Bronchial Lymph Node	Plasma cell hyperplasia							
	minimal:	**2/15**	**0/1**	**0/0**	**7/17**			
	Lymphoid atrophy							
	minimal:	**3/15**	**0/1**	**0/0**	**4/17**			
	Lymphocytolysis							
	minimal:	**1/15**	**0/1**	**0/0**	**2/17**			
	Congestion							
	minimal:	**2/15**	**0/1**	**0/0**	**3/17**			
	Pigment deposit							
	minimal:	**3/15**	**0/1**	**0/0**	**2/17**			
Mesenteric Lymph Node	Increased mast cell							
	minimal:	**2/19**	**8/20**	**6/20**	**6/20**	**0/10**	**0/10**	**0/9**
	Lymphoid atrophy							
	minimal:	**5/19**	**13/20**	**16/20**	**14/20**	**3/10**	**6/10**	**6/9**
	slight:	**0/19**	**6/20**	**3/20**	**5/20**	**0/10**	**1/10**	**0/9**
	moderate:	**0/19**	**0/20**	**0/20**	**1/20**	**0/10**	**1/10**	**0/9**
	Hemangiosarcoma							
	minimal:	**0/19**	**0/20**	**0/20**	**0/20**	**0/10**	**0/10**	**1/9**
Mandibular Lymph Node	Lymphoid atrophy							
	minimal:	**0/19**	**3/19**	**6/19**	**6/20**	**1/10**	**0/0**	**1/8**
	slight:	**0/19**	**0/19**	**0/19**	**0/20**	**0/10**	**0/0**	**0/8**
	Plasma cell hyperplasia							
	minimal:	**3/19**	**1/19**	**1/19**	**3/20**	**2/10**	**0/0**	**1/8**
	slight:	**5/19**	**0/19**	**0/19**	**9/20**	**2/10**	**0/0**	**2/8**
	moderate:	**0/19**	**0/19**	**0/19**	**0/20**	**2/10**	**0/0**	**0/8**
Salivary glands	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/20**	**0/21**	**1/10**	**0/0**	**0/9**
	slight:	**0/20**	**0/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**
	Artery inflammation							
	minimal:	**0/20**	**0/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**

Tissue	Findings	Main Study 0	Main Study 0.3	Main Study 1.5	Main Study 7.5	Recovery 0	Recovery 1.5	Recovery 7.5
Salivary glands	Inflammatory focus							
	minimal:	**0/20**	**0/1**	**0/20**	**0/21**	**0/10**	**0/0**	**0/9**
	Acinar cell hyperplasia							
	minimal:	**0/20**	**0/1**	**0/20**	**0/21**	**0/10**	**0/0**	**0/9**
Tongue	Atrophy							
	minimal:	**0/20**	**0/1**	**0/19**	**1/19**	**0/10**	**0/0**	**0/9**
	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/19**	**2/19**	**0/10**	**0/0**	**0/9**
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/19**	**2/19**	**0/10**	**0/0**	**0/9**
Small intestine	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/20**	**2/20**			
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/20**	**2/20**			
	Artery inflammation							
	minimal:	**0/20**	**0/1**	**0/20**	**1/20**			
Large intestine	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/20**	**1/20**			
	Artery wall thickening							
	minimal:	**0/20**	**0/1**	**0/20**	**2/20**			
	Artery inflammation							
	minimal:	**0/20**	**0/1**	**0/20**	**2/20**			
Pancreas	Acinar cell atrophy							
	minimal:	**3/19**	**0/1**	**3/20**	**5/21**	**3/10**	**0/0**	**1/9**
	slight:	**0/19**	**1/1**	**0/20**	**0/21**	**0/10**	**0/0**	**0/9**
	Metaplasia							
	minimal:	**0/19**	**0/1**	**1/20**	**0/21**	**0/10**	**0/0**	**0/9**
	Fibrinoid necrosis							
	minimal:	**0/19**	**0/1**	**0/20**	**2/21**	**0/10**	**0/0**	**0/9**
	Artery wall thickening							
	minimal:	**0/19**	**0/1**	**0/20**	**2/21**	**0/10**	**0/0**	**0/9**
	slight:	**0/19**	**0/1**	**0/20**	**1/21**	**0/10**	**0/0**	**0/9**
	Artery inflammation							
	minimal:	**0/19**	**0/1**	**0/20**	**3/21**	**0/10**	**0/0**	**0/9**
	Peri-islet pigment							
	minimal:	**0/19**	**0/1**	**0/20**	**2/21**	**0/10**	**0/0**	**2/9**
	Interstitial pigment							
	minimal:	**0/19**	**0/1**	**0/20**	**0/21**	**0/10**	**0/0**	**2/9**
Prostate	Inflammatory focus							
	minimal:	**1/20**	**0/20**	**0/20**	**1/21**	**1/10**	**0/10**	**1/9**
	Decreased secretory prod[n]							
	minimal:	**0/20**	**6/20**	**8/20**	**12/21**	**0/10**	**2/10**	**2/9**
Testes	Tubular atrophy							
	minimal:	**0/20**	**0/1**	**0/20**	**2/21**	**0/10**	**1/1**	**0/9**
	slight:	**0/20**	**1/1**	**0/20**	**0/21**	**0/10**	**1/1**	**0/9**
	moderate:	**0/20**	**0/1**	**0/20**	**0/21**	**2/10**	**1/1**	**0/9**
	marked:	**0/20**	**0/1**	**0/20**	**0/21**	**0/10**	**1/1**	**1/9**
	Spermatic giant cells							
	minimal:	**0/20**	**1/1**	**0/20**	**1/21**	**0/10**	**1/1**	**1/9**
	Fibrinoid necrosis							
	minimal:	**0/20**	**0/1**	**0/20**	**0/21**	**0/10**	**1/1**	**0/9**
	slight:	**0/20**	**0/1**	**0/20**	**2/21**	**0/10**	**1/1**	**0/9**

Tissue	Findings	Main Study 0	0.3	1.5	7.5	Recovery 0	1.5	7.5
Testes	Artery wall thickening							
	minimal:	0/20	0/1	0/20	0/21	0/10	1/1	0/9
	slight:	0/20	0/1	0/20	2/21	0/10	1/1	0/9
	Artery inflammation							
	minimal:	0/20	0/1	0/20	2/21	0/10	1/1	0/9

Summary of major female microscopic findings

Tissue	Findings	Main Study 0	0.3	1.5	7.5	Recovery 0	1.5	7.5
Eyes	Inflammation							
	minimal:	0/20	0/1	0/1	1/22			
	Conjunctivitis							
	minimal:	0/20	0/1	0/1	1/22			
	Cataracts							
	minimal:	0/20	0/1	0/1	0/22			
	slight:	0/20	0/1	0/1	1/22			
Lungs	Lymphoid cuffing							
	minimal:	3/20	2/20	4/21	0/20	4/10	6/9	4/8
	slight:	1/20	0/20	0/21	0/20	1/10	1/9	0/8
	Inflammatory focus							
	minimal:	2/20	0/20	1/21	0/20	0/10	0/9	0/8
	Smooth muscle hypertrophy							
	minimal:	0/20	3/20	16/21	20/20	0/10	1/9	4/8
	slight:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	Osseous metaplasia							
	minimal:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	Hemorrhage							
	minimal:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	moderate:	0/20	1/20	0/21	0/20	0/10	0/9	0/8
	Edema							
	minimal:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/20	0/10	0/9	0/8
	moderate:	0/20	0/20	0/21	1/20	0/10	0/9	0/8
	Alveolar macrophage							
	minimal:	1/20	0/20	0/21	0/20	1/10	1/9	0/8
	Congestion							
	present:	0/20	1/20	1/21	2/20	0/10	0/9	0/8
Kidneys	Basophilic tubules							
	minimal	1/20	3/20	14/21	16/22	2/10	7/9	8/8
	slight:	0/20	0/20	1/21	4/22	0/10	0/9	0/8
	moderate:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	marked:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Tubular hypertrophy							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Tubular hyperplasia							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Hyaline cast							
	minimal	1/20	1/20	2/21	0/22	2/10	2/9	1/8
	slight:	0/20	0/20	0/21	0/22	0/10	1/9	1/8

Tissue	Findings	Main Study 0	Main Study 0.3	Main Study 1.5	Main Study 7.5	Recovery 0	Recovery 1.5	Recovery 7.5
Kidneys	Hyaline droplets							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Artery wall thickening							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Fibrinoid necrosis							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	moderate:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Hemorrhage							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Cyst							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Mineralization							
	minimal:	9/20	11/20	12/21	9/22	5/10	6/9	3/8
	slight:	2/20	4/20	4/21	7/22	3/10	1/9	0/8
	moderate:	5/20	1/20	0/21	1/22	0/10	0/9	0/8
	severe:	0/20	0/20	0/21	0/22	1/10	0/9	0/8
Spleen	Increased hematopoiesis							
	minimal:	0/20	2/20	7/21	6/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	3/22	1/10	0/9	0/8
	moderate	0/20	1/20	0/21	0/22	8/10	8/9	1/8
	severe:	0/20	0/20	0/21	0/22	1/10	1/9	7/8
	Lymphoid atrophy							
	minimal:	0/20	12/20	15/21	6/22	0/10	0/9	4/8
	slight:	0/20	6/20	6/21	16/22	0/10	0/9	0/8
	Artery wall thickening							
	minimal:	0/20	0/20	0/21	1/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Fibrinoid necrosis							
	minimal:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	slight:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
	Hemangiosarcoma							
	present:	0/20	0/20	0/21	0/22	0/10	0/9	0/8
Thymus	Atrophy of cortex							
	minimal:	0/20	0/20	0/21	0/21	0/10	0/9	0/8
	slight:	0/20	1/20	2/21	1/21	0/10	0/9	4/8
	moderate:	0/20	16/20	16/21	17/21	0/10	0/9	4/8
	severe:	0/20	3/20	3/21	3/21	0/10	0/9	0/8
	Enlarged medulla:							
	minimal:	0/20	0/20	0/21	0/21	0/10	0/9	0/8
	slight:	0/20	0/20	1/21	0/21	0/10	0/9	0/8
	moderate:	0/20	17/20	17/21	18/21	0/10	0/9	8/8
	severe:	0/20	3/20	3/21	3/21	0/10	0/9	0/8
Bronchial Lymph Node	Plasma cell hyperplasia							
	minimal:	0/15	0/18	0/16	0/16	0/9	0/0	0/8
	Lymphoid atrophy	0/15	0/18	0/16	0/16	0/9	0/0	0/8
	minimal:	1/15	8/18	11/16	13/16	1/9	0/0	0/8
	Lymphocytolysis							
	minimal:	0/15	0/18	0/16	0/16	0/9	0/0	0/8

Tissue	Findings	Main Study 0	Main Study 0.3	Main Study 1.5	Main Study 7.5	Recovery 0	Recovery 1.5	Recovery 7.5
Bronchial Lymph Node	Congestion							
	minimal:	3/15	0/18	2/16	2/16	0/9	0/0	0/8
	Pigment deposit							
	minimal:	6/15	0/18	0/16	7/16	0/9	0/0	0/8
Mesenteric Lymph Node	Increased mast cell							
	minimal:	1/20	6/20	6/21	5/22	2/9	0/8	2/8
	Lymphoid atrophy							
	minimal:	2/20	14/20	13/21	7/22	0/9	0/8	5/8
	slight:	0/20	4/20	8/21	12/22	0/9	0/8	0/8
	moderate:	0/20	0/20	0/21	1/22	0/9	0/8	0/8
	Hemangiosarcoma							
	minimal:	0/20	0/20	0/21	0/22	0/9	0/8	0/8
Mandibular Lymph Node	Lymphoid atrophy							
	minimal:	1/20	4/19	3/20	11/21	0/9	0/0	0/7
	slight:	0/20	0/19	0/20	1/21	0/9	0/0	0/7
	Plasma cell hyperplasia							
	minimal:	4/20	2/19	6/20	6/21	7/9	0/0	5/7
	slight:	7/20	4/19	1/20	13/21	1/9	0/0	2/7
	moderate:	5/20	0/19	0/20	0/21	0/9	0/0	0/7

Immune function:

Approximately 4 weeks before necropsy, 6 F in each group were treated to assess the sheep red blood cell (SRBC) immune response. Treatment during the induction phase was performed by i.p. injection of SRBC suspension or vehicle alone. The challenge was performed 3 weeks later by i.v. injection of SRBC (same concentration and volume). At necropsy, blood samples were taken and processed for SRBC agglutination in serum, as an indicator for SRBC-specific IgM antibodies. The results of this investigation were reported separately (Study № PCS-r991052a).

All animals generated SRBC specific IgM, but in FTY720 treated animals there was a decrease in SRBC specific IgM, however, there was no dose dependency.

Toxicokinetics

Blood samples were taken on Day 1-2 and in Week 22 at 1, 2, 4, 7, 12 and 24 hours.

Plasma exposure to FTY720 was dose-proportional with accumulation over the course of the study, $AUC_{0\text{-}24h}$, in M at the HD, was 4,439 and 9,342 ng*h/ml and 4,664 and 9,772 ng*h/ml, in F at the HD, at Day 1-2 and Week 22, respectively. C_{max} values were 231 and 450 ng/ml in M at the HD and 229 and 568 ng/ml in F at the HD at Day 1-2 and Week 22, respectively. T_{max} values were 4-12 hours.

Table of toxicokinetic parameters (Sponsor's)

Period	Day 1 to 2			Week 22		
Dose[mg/kg/day]	0.3	1.5	7.5	0.3	1.5	7.5
Males						
t_{max}	7.00	4.00	12.0	4.00	4.00	12.0
C_{max}	9.22	37.1	231	21.9	131	450
$C_{max/dose}$ [1)]	30.7	24.7	30.8	73.0	87.3	60.0
$AUC_{(0-24h)}$ [2)]	154	712	4439	418	2597	9342
$AUC_{(0-24h)/dose}$ [3)]	513	474	592	1393	1731	1246
Females						
t_{max}	4.00	4.00	12.0	4.00	4.00	4.00
C_{max}	9.49	39.3	229	24.6	123	568
$C_{max/dose}$ [1)]	31.6	26.2	30.5	82.0	82.0	75.7
$AUC_{(0-24h)}$ [2)]	151	820	4664	456	1993	9772
$AUC_{(0-24h)/dose}$ [3)]	502	547	622	1521	1329	1303

Units: 1) (ng/mL)/(mg/kg/day); 2) h.ng/mL; 3) (h.ng/mL)/(mg/kg/day)

Stability and Homogeneity

FTY720 was stable and homogenously distributed. Pre-treatment samples from groups 2 and 4 had ranges of 1.5% and 3.4% from top to bottom and were stable for up to 6 hours.

Study title: Y-32720: One-month oral toxicity study in dogs

Study no.: PCS-r7010

Study report location: EDR: 4.2.3.2.1

Conducting laboratory and location: (b) (4)

Date of study initiation: 2 February 1995

GLP compliance: Yes (1982, 1988)

QA statement: Yes (Dated: 7 Apr 1995, amended 14 Sep 1998)

Drug, lot #, and % purity: Y-32720, Lot I, 100.1%

Key Study Findings

- Lung weight was increased and histopathological findings included pneumonia, hemorrhage, edema, macrophage accumulation and fibrosis. Lymphoid organ were atrophied at doses equal to and greater than 0.01 mg/kg.
- The NOAEL was 0.01 mg/kg/day as lung weight was increased and histopathological changes observed at doses greater than 0.01 mg/kg/day.

Methods

Doses:	0, 0.001, 0.01, 0.1 and 1 mg/kg/day (C, LD, MLD, MHD and HD)
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	Gelatin capsules with 50 ml tap water
Formulation/Vehicle:	30 mg/kg Lactose
Species/Strain:	Dog/Beagle
Number/Sex/Group	
Main study:	3
Recovery:	2 for 0, 0.01 and 0.1 groups
Age:	7-8 months at start of treatment
Weight	
Males:	8.5-10.9 g
Females:	7.1-10.3 g

Observations and Results

Mortality

There were no deaths.

Clinical Signs

Observations were performed daily pre-treatment and twice or more daily during treatment and recovery.

One F at the HD had signs of salivation and decreased locomotion on Day 21, and 1 M at the MLD and 1 F at the HD showed signs of vomiting, otherwise there were no drug-related changes.

Body Weights

Body weights were recorded once weekly pre-treatment, during treatment and recovery.

One F at the HD dog lost 0.8 kg (8%) in Week 3; otherwise, there were no drug-related changes in body weight.

Food Consumption

Food consumption was determined twice a week from pre-treatment, during treatment and recovery.

There were no drug-related changes in food consumption.

Ophthalmoscopy and hearing

Examinations of all rats were performed 2 weeks pre-treatment and in Week 4 during treatment and at the end of recovery.

No ophthalmoscopy data were submitted. It was reported that there were no ophthalmoscopic findings.

Hematology
Blood samples were taken from all animals; 1 week pre-treatment and in Weeks 2 and 4 of treatment and Week 2 and 4 of recovery. The following parameters were assessed: erythrocyte, leukocyte and platelet counts, HGB, HCT and blood coagulation.

In M at Week 4, WBCs were decreased in all treatment groups by 18-60% and lymphocytes were decreased by 39-94%. At Week 4, F had decreased WBCs at the MLD, MHD and HD by 35-47% and decreased lymphocytes by 83-92%. There was reversal of levels by Week 4 of recovery.

Clinical Chemistry
Blood samples were taken from all animals 1 week pre-treatment and in Weeks 2 and 4 of treatment and Week 2 of recovery. The following parameters were assessed: GOT, GPT, ALP, CPK, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

In M at Week 4, there was a decrease from pre-treatment levels at the MLD in CPK (22%), at the MHD in CPK (33%), GPT (15%), phospholipids (%), total protein (4%) and α_2-globulin (21%) and at the HD in CPK (37%), GPT (26%), phospholipids (%), total protein (13%), α_1-globulin (33%), α_2-globulin (25%) and A/G ratio (17%). MHD male changes in GPT, CPK, phospholipids and total protein were reversed, while α_2-globulin levels remained increased during recovery.

In F, there was a decrease from pre-treatment levels at the LD in CPK (30%) and total cholesterol (8%), at the MLD in CPK (26%) and total cholesterol (12%), at the MHD in CPK (47%), total cholesterol (18%), GPT (19%), phospholipids (16%), total protein (6%), α_2-globulin (19%) and A/G ratio (13%) and at the HD in CPK (26%), total cholesterol (35%), GPT (29%), phospholipids (31%), total protein (20%), α_2-globulin (27%) and A/G ratio (9%). In HMD F, changes in GPT, total cholesterol, phospholipids, total protein and A/G ratio were reversed; CPK levels continued to decrease in all recovery groups, while α_2-globulin levels remained increased during recovery.

Urinalysis
Urine samples, collected over a 20 hour period, were taken from all animals pre-treatment, in weeks 2 and 4 of treatment and week 4 of recovery. The following parameters were assessed: volume, specific gravity, pH, protein, occult blood, glucose, ketone bodies, urobilinogen and bilirubin, sodium, potassium and chlorine, urine sediment was examined microscopically.

There appeared no drug-related treatment changes in urinalysis at Week 4.

Gross Pathology
Gross pathology was assessed in all animals.

Gross findings of foamy contents were observed in the lungs of M; 2 MHD and 3 HD, and dark red discoloration of the lungs were observed in 2 M and 2 F at the MHD and in all M and 2 F at the HD. The lung was not collapsed and was enlarged in 1 M and 1 F at the MHD.

Organ Weights
At necropsy the following organs were weighed: brain, heart, lungs, liver, kidneys, trachea, spleen, pituitary gland, adrenals, thyroid glands, pancreas, submandibular glands, thymus, prostate, ovary, uterus and testes.

In M, there was a dose-dependent increase in relative weights of lungs (6%, 28% and 86%) and adrenal glands (4%, 23% and 10%) and a decrease in pituitary gland (5%, 14% and 17%) at the MLD, MHD and HD, respectively. The organ weights recovered.

In F, there was an increase in relative weights of MLD, MHD and HD liver (17%, 4% and 13%), lungs (6%, 33% and 92%) and adrenal glands (4%, 7% and 13%), compared to controls. At the HD, there was no recovery in liver weight (18%), but recovery in lungs and adrenal was observed.

Histopathology
At necropsy, the following organs were prepared for histopathology examination: liver, kidneys, lungs, trachea, heart, spleen, pancreas, brain, pituitary gland, spinal cord, adrenals, thyroid and parathyroid glands, thymus, submandibular and mesenteric lymph nodes, parotid, sublingual and submandibular glands, femur and sternum bone, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, gallbladder, aorta, prostate, testes, epididymis, ovary, uterus, vagina, mammary glands, skin, tongue and lacrimal glands. Eye balls were fixed in formaldehyde-glutaraldehyde solution.

Adequate Battery: Yes
Peer Review: No

There was lymphoid organ atrophy at doses equal to and greater than MLD that was consistent with a pharmacological action of FTY720. The other microscopic finding was in the lungs and included pneumonia, hemorrhage, edema, macrophage accumulation and fibrosis.

Summary of major microscopic findings

		MALE						FEMALE					
		Main				Rec.		Main				Rec.	
Tissue	**Findings**	**0.001**	**0.01**	**0.1**	**1**	**0.01**	**0.1**	**0.001**	**0.01**	**0.1**	**1**	**0.01**	**0.1**
Lungs	Bronchiolar alteration												
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/3	1/2	0/2
	slight:	0/3	0/3	1/3	0/3	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2
	moderate:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/3	1/2	0/2
	Pneumonia												
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	2/3	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	1/3	1/3	0/2	0/2
	moderate:	0/3	0/3	0/3	2/3	0/2	0/2	1/3	0/3	0/3	0/3	0/2	0/2
	Hemorrhage:												
	very slight:	0/3	0/3	0/3	2/3	0/2	0/2	1/3	0/3	0/3	1/3	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2
	Edema												
	very slight:	0/3	0/3	0/3	1/3	0/2	0/2	1/3	0/3	0/3	1/3	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2
	moderate:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2
	Macrophage accumulation												
	very slight:	0/3	0/3	2/3	0/3	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2
	slight:	0/3	0/3	1/3	0/3	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2
	moderate:	0/3	0/3	0/3	3/3	0/2	0/2	0/3	0/3	0/3	3/3	0/2	0/2
	Subpleural fibrosis												
	very slight:	0/3	0/3	1/3	0/3	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/3	1/2	0/2
	Inflammatory cell infiltration												
	very slight:	0/3	0/3	0/3	3/3	0/2	0/2	1/3	0/3	1/3	0/3	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2
Kidneys	Tubular regeneration												
	very slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2
	Interstitial cell infiltration												
	very slight:	0/3	0/3	0/3	0/3	1/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2
Spleen	White pulp atrophy												
	very slight:	0/3	0/3	2/3	0/3	0/2	0/2	0/3	0/3	2/3	1/3	0/2	0/2
	slight:	0/3	0/3	1/3	3/3	0/2	0/2	0/3	0/3	1/3	2/3	0/2	0/2
Thymus	Atrophy of cortex												
	very slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2
	Enlarged medulla												
	very slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2
Mesenteric Lymph Node	Atrophy												
	very slight:	0/3	2/3	3/3	2/3	0/2	0/2	0/3	2/3	2/3	2/3	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2
Submandibular Lymph Node	Atrophy												
	very slight:	0/3	2/3	3/3	2/3	0/2	0/2	0/3	2/3	2/3	2/3	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2

Rec. - recovery.

Phagocytosis test of leukocytes (non-GLP).

Blood samples were taken from all M at 8 hours post-dose on Day 1, Week 2 and Week 4 of recovery.

Phagocytosis of yeast particles was inhibited at the HD on Day 1, at the MLD, MHD and HD in Week 2; however, there was no inhibition after 4 weeks at any dose compared with control.

Microbial examination of diseased lung (non-GLP).
A small piece of lung was taken and examined from 2 F at the MHD and 1 F at the HD.

There were no differences in the number of microbes isolated from each piece of lung.

Toxicokinetics
Blood samples were taken from MLD, MHD and HD group animals on Day 1; at pre-dose, 8 and 24 hours post-dose, in Weeks 2 and 4 of treatment at 8 and 24 hours and in Weeks 2 and 4 of recovery at 8 and 24 hours.

No general sex differences in plasma levels at 8 and 24 hours post-dose were observed. Plasma levels increased dose-proportionally. Accumulation of 1.9-3.0-fold occurred at Week 4. Very low levels (0.1-0.2 ng/ml) of FTY720 were still detected at Week 4 of the recovery period.

Table of toxicokinetic parameters (Sponsor's)

Sex	Dose (mg/kg)	Animal No.	Blood concentration (ng/ml)						
			1st day			2 weeks		4 weeks	
			Before	8 h	24 h	8 h	24 h	8 h	24 h
Male	0.01	3101	ND	0.3	0.2	0.5	0.4	0.6	0.3
		3102	ND	0.3	0.2	0.4	0.3	0.7	0.4
		3103	ND	0.3	0.1	0.4	0.6	0.5	0.3
		3104	ND	0.3	0.2	0.5	0.2	0.6	0.4
		3105	ND	0.3	0.2	0.8	0.5	0.9	0.6
		Mean	-	0.3	0.2	0.5	0.4	0.7	0.4
		± SD	-	0.0	0.0	0.2	0.2	0.2	0.1
	0.1	4101	ND	3.0	2.1	6.2	4.5	8.8	5.0
		4102	ND	4.6	2.4	5.5	2.9	6.1	3.3
		4103	-a)	3.3	2.0	5.5	3.1	5.3	2.8
		4104	ND	2.9	2.1	6.0	3.5	6.0	4.0
		4105	ND	3.5	2.3	6.3	3.7	7.9	5.1
		Mean	-	3.5	2.2	5.9	3.5	6.8	4.0
		± SD	-	0.7	0.2	0.4	0.6	1.5	1.0
	1	5101	ND	27.7	11.6	29.6	13.3	37.9	18.2
		5102	ND	21.7	10.5	34.9	17.7	49.0	20.6
		5103	ND	19.2	14.1	41.6	22.2	57.2	24.9
		Mean	-	22.9	12.1	35.4	17.7	48.0	21.2
		± SD	-	4.4	1.8	6.0	4.5	9.7	3.4
Female	0.01	3201	ND	0.3	0.2	0.6	0.4	0.8	0.5
		3202	ND	0.3	0.2	0.5	0.3	1.0	0.3
		3203	ND	0.2	0.2	0.5	0.2	0.5	0.3
		3204	ND	0.2	0.2	0.5	0.3	0.9	0.5
		3205	ND	0.3	0.1	0.8	0.4	1.2	0.6
		Mean	-	0.3	0.2	0.6	0.3	0.9	0.4
		± SD	-	0.1	0.0	0.1	0.1	0.3	0.1
	0.1	4201	ND	4.2	2.3	5.9	4.6	6.1	4.2
		4202	ND	2.9	1.6	4.6	3.0	4.8	3.1
		4203	ND	2.6	2.2	6.3	3.8	7.0	4.1
		4204	ND	3.3	2.6	8.1	4.6	7.1	4.8
		4205	ND	3.1	1.9	3.2	1.9	5.1	3.1
		Mean	-	3.2	2.1	5.6	3.6	6.0	3.9
		± SD	-	0.6	0.4	1.8	1.1	1.1	0.7
	1	5201	ND	23.4	13.9	54.6	23.4	51.9	32.8
		5202	ND	34.1	18.3	69.5	38.2	90.7	45.1
		5203	ND	30.2	19.2	81.6	54.3	95.6	77.5
		Mean	-	29.2	17.1	68.6	38.6	79.4	51.8
		± SD	-	5.4	2.8	13.5	15.5	23.9	23.1

ND : Not detected.
a) : Operational failure in GC/MS.

Stability and Homogeneity
There were no data presented.

Study title: Y-32720: One-month oral toxicity study in dogs (additional high-dose study)

Study no.:	PCS-r7148 and DMPK-r6988
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	10 August 1994
GLP compliance:	Yes ([redacted] (b) (4), 1982, 1988)
QA statement:	Yes (Dated: 8 Dec 1995, amended 14 Sep 1998)
Drug, lot #, and % purity:	Y-32720, Lot M, 100.3%

Key Study Findings

- White blood cells and lymphocyte counts were decreased in all treated groups and lymphoid organ atrophy was observed.
- Lung weight was increased in all groups and histological findings of subpleural fibrosis, focal bronchiolar alterations, alveolar macrophage infiltration and pneumonia were observed at 10 and 30 mg/kg/day.
- Other histological findings included vascular wall thickening, and perivascular focal perimysial fibrosis of the left ventricular papilla in the heart of all treated groups. Animals at 30 mg/kg/day had perivascular monocyte infiltration of the brain, peripheral nerve degeneration of the heart, ganglion cell vacuolation of Auerbach's plexus and vacuolation of anterior pituitary cells.
- A NOAEL was not established.

Methods

Doses:	0, 3, 10 and 30 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	Gelatin capsules with 50 ml tap water
Formulation/Vehicle:	Empty capsule
Species/Strain:	Dog/Beagle
Number/Sex/Group	
Main study:	3
Recovery:	2 for control, 10 and 30 mg/kg groups
Age:	7 months at start of treatment
Weight	
Males:	7.7-10.2 g
Females:	7.3-8.8 g
Deviation from study protocol:	One control F (1205) received 3 mg/kg in Week 2 that resulted in a decrease in lymphocyte counts in Week 2 blood samples; there was no abnormality in this animal in Week 4 blood samples.

Observations and Results

Mortality

There were no deaths.

Clinical Signs

Observations were performed twice daily on weekdays, at pre-treatment, 3 times or more daily during treatment and 2 times or more daily during recovery.

Loose stools and diarrhea were observed in all groups; 1 control M, 1 LD M, 2 LD F, all MD M, 4 MD F and all HD animals. Salivation and vomiting was observed in all HD animals and vomiting was occasionally observed in LD and MD groups.

Body Weights

Body weights were recorded weekly throughout the study.

Decrease in weight gain occurred in M (0.5, 0.6, 0.3 and -1 kg) and in F (0.4, 0.3, 0.3, and -0.3 kg) at control, LD, MD and HD, respectively. There was recovery in weights after the recovery period.

Food Consumption

Food consumption was determined weekly throughout the study.

There was decreased food consumption in Week 1 in 1, 1 and 1 M at the LD, MD and HD, respectively. A slight decrease in food consumption continued in M at the LD and MD, with a large decrease seen in M at the HD that decreased to 57% of control values. There was decreased food consumption in 1, 2 and 3 F at the LD, MD and HD. A slight decrease in food consumption continued in F at the LD and MD with a larger decrease in F at the HD. There was recovery of food consumption during the recovery period.

Ophthalmoscopy and hearing

Examinations of all rats were performed in 2 weeks pre-treatment and in Week 4 during treatment and Week 4 during recovery.

No ophthalmoscopy data were submitted. It was reported that there were no ophthalmoscopic findings.

Hematology

Blood samples were taken from all animals 1 week pre-treatment, in Weeks 2 and 4 during treatment and Weeks 2, 4, 6 and 8 during recovery. The following parameters were assessed: erythrocyte, leukocyte and platelet counts, HGB and HCT, MCV, MCH, MCHC, reticulocytes, PT and APTT.

RBC parameters in animals at the HD were slightly increased. WBCs were decreased in all treated groups by 25-60% with lymphocytes decreased by 81-93%. Eosinophils were decreased in all treated groups and neutrophils decreased at the HD. During

recovery, these changes started to reverse for WBCs, lymphocytes and neutrophils, but not eosinophils.

Clinical Chemistry

Blood samples were taken from all animals 1 week pre-treatment, in weeks 2 and 4 during treatment and weeks 2, 4, 6 and 8 during recovery. The following parameters were assessed: GOT, GPT, ALP, CPK, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

In M, observations at 4 weeks included a decrease from pre-treatment levels at the LD in total cholesterol (32%), phospholipids (33%), total protein (21%) and calcium (7%), at the MD in total cholesterol (22%), phospholipids (25%), total protein (19%), calcium (6%), ALP (31%) and γ-globulin (21%) and at the HD in total cholesterol (20%), phospholipids (24%), total protein (19%), calcium (7%), ALP (35%) and γ-globulin (32%). There were increases from pre-treatment levels at the LD in triglycerides (8%), α_1-globulin (26%) and α_2-globulin (21%), at the MD in triglycerides (58%), α_1-globulin (22%) and α_2-globulin (17%) and at the HD in GPT (338%), potassium (10%), triglycerides (29%), α_1-globulin (20%) and α_2-globulin (32%). At the HD, changes in GPT, triglycerides, total cholesterol, phospholipids, total protein, α_1-globulin, calcium recovered, ALP levels continued to decrease in all recovery groups, whilst potassium, α_2-globulin and γ-globulin levels remained changed during recovery.

In F, decreases from pre-treatment levels included ALP (26%), total cholesterol (28%), phospholipids (27%), total protein (16%), A/G ratio (20%), and calcium (3%) and increases included α_1-globulin (20%), α_2-globulin (38%) and potassium (15%) at the LD. At the MD, changes included decreases in ALP (36%), total cholesterol (22%), phospholipids (23%), total protein (14%), A/G ratio (17%), γ-globulin (5%) and calcium (6%) and increases in α_1-globulin (19%), α_2-globulin (30%) and potassium (11%). At the HD, changes included decreases in ALP (38%), total cholesterol (21%), phospholipids (22%), total protein (16%), A/G ratio (12%), γ-globulin (26%) and calcium (5%) and increases in α_1-globulin (28%), α_2-globulin (45%) and potassium (16%). In F, changes in ALP, total cholesterol, phospholipids, total protein, α_1-globulin, potassium, and calcium were reversed, while A/G ratio α_2-globulin and γ-globulin levels remained changed during recovery.

Urinalysis

Urine samples, collected over a 20-hour period, were taken from all animals pre-treatment, in weeks 2 and 4 of treatment and weeks 4 and 8 of the recovery. The following parameters were assessed: volume, specific gravity, pH, protein, occult blood, glucose, ketone bodies, urobilinogen and bilirubin, sodium, potassium and chlorine, urine sediment was examined for erythrocytes, leukocytes, small round cells, squamous cells and casts.

In M, urine volume was decreased at the HD and sodium, potassium and chloride was decreased in all HD animals.

Gross Pathology
Gross pathology was assessed in all animals.

Dark red lungs were observed in 1, 1 and 1 M and in 1, 2 and 1 F at the LD, MD and HD, respectively. The lung was not collapsed and was enlarged in several animals from all treatment groups. Small thymuses were observed in all M and 1 F at the HD and small testes in 1 M at the HD.

Organ Weights
At necropsy, the following organs, were weighed: brain, heart, lungs, liver, kidneys, spleen, hypophysis, adrenals, thyroid glands, pancreas, submandibular glands, thymus, prostate, ovary, uterus and testes.

In M, relative weights of liver (9%) and lungs (110%) were increased and the thymus (23%) was decreased at the LD. At the MD, liver (27%), lung (116%) and brain (6%) weights were increased. At the HD, liver (32%), lung (22%), brain (36%), adrenal glands (46%) and pancreas (32%) weights were increased and the thymus weight (74%) was decreased at the HD. In addition, 1 M at the HD had a large pituitary gland. All organ weights recovered.

In F, relative weights of liver (27%), lungs (113%), heart (4%) and brain (10%) were increased and the thymus weight (23%) was decreased at the LD. At the MD, liver (23%), lungs (106%), heart (11%) and brain (7%) weights were increased. At the HD liver (32%), lungs (86%), heart (15%) and brain (27%), adrenal glands (47%), pancreas (59%) and ovary (114%) weights were increased and thymus weight (74%) was decreased. In addition, 1 F at the HD had a large uterus. All organ weights recovered.

Histopathology
At necropsy the following organs were prepared for histopathology examination: liver, kidneys, lungs, trachea, heart, spleen, pancreas, brain, pituitary gland, spinal cord, adrenals, thyroid and parathyroid glands, thymus, submandibular and mesenteric lymph nodes, parotid, sublingual and submandibular glands, femur and sternum bone, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, gallbladder, aorta, prostate, epididymis, ovary, uterus, vagina, mammary glands, skin, tongue and lacrimal glands. Tissues were fixed in 10% formalin, except for testes that were fixed in Bouin's solution and eye balls that were fixed in formaldehyde-glutaraldehyde solution. In addition, sections of adrenals were stained with Grimelius method and sections from heart, brain and stomach were stained with Kluver-Barrera's method.

Adequate Battery: Yes
Peer Review: No

Lymphoid atrophy was observed in all treated groups, consistent with the pharmacological action of FTY720. In the lungs, subpleural fibrosis, focal bronchiolar alterations, alveolar macrophage infiltration and pneumonia were observed at MD and

HD. Vascular wall thickening and perivascular focal perimysial fibrosis of the left ventricular papilla occurred in the heart of all treated groups. Vacuolation and fibrosis of the adrenal medulla were observed at the MD and HD. HD animals had findings of perivascular monocyte infiltration of the brain (gray matter), peripheral nerve degeneration (axon and Schwann cells) of the heart, ganglion cell vacuolation of Auerbach's plexus (stomach), vacuolation of anterior pituitary cells, brown pigmentation of Kupffer cells (liver), hypospermatogenesis in the tubules of the testes and hypospermia in the epididymides were observed. After 8 weeks recovery, the findings in the lymphoid organs, adrenal glands and heart remained, but with decreased severity.

Summary of major microscopic findings

		MALE					FEMALE				
		Main			Rec.		Main			Rec.	
Tissue	Findings	3	10	30	10	30	3	10	30	10	30
Brain	Peri. mononuclear infiltration										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	2/3	0/2	0/2
	moderate:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
	Mononuclear infiltration										
	very slight:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
	slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
Spinal cord	Peri. mononuclear infiltration										
	very slight:	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Peripheral nerves	Degeneration heart nerve fibers										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	2/3	0/2	0/2
	Degeneration Auerbach plexus										
	very slight:	0/3	0/3	2/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
	slight:	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Pituitary gland	Glandular cell vacuolation										
	very slight:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Adrenal gland	Fascicular cell vacuolation										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	1/2	0/2
	Medullary cell vacuolation										
	very slight:	0/3	0/3	0/3	0/2	1/2	0/3	1/3	0/3	1/2	0/2
	Slight:	0/3	2/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	moderate:	0/3	0/3	3/3	0/2	0/2	0/3	1/3	3/3	0/2	0/2
	Decreased medullary cell										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	1/2	0/2
	Slight:	0/3	0/3	0/3	0/2	2/2	0/3	0/3	0/3	0/2	0/2
	moderate:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	2/2
	severe:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	3/3	0/2	0/2
	Medulla fibrosis										
	very slight:	0/3	1/3	0/3	0/2	0/2	0/3	0/3	0/3	1/2	0/2
	slight:	0/3	0/3	1/3	0/2	2/2	0/3	0/3	0/3	0/2	1/2
	moderate:	0/3	0/3	2/3	0/2	0/2	0/3	0/3	3/3	0/2	1/2
Heart	Myocardial degeneration/necrosis										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
	Cell infiltration										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	2/3	0/2	0/2	1/3	0/3	0/3	0/2	0/2

Tissue	Findings	MALE Main 3	MALE Main 10	MALE Main 30	MALE Rec. 10	MALE Rec. 30	FEMALE Main 3	FEMALE Main 10	FEMALE Main 30	FEMALE Rec. 10	FEMALE Rec. 30
Heart	Peri. fibrosis										
	very slight:	0/3	1/3	1/3	0/2	0/2	0/3	1/3	1/3	0/2	0/2
	slight:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Thickening of vessel wall										
	very slight:	1/3	1/3	2/3	1/2	0/2	1/3	2/3	0/3	0/2	1/2
	slight:	1/3	2/3	0/3	0/2	0/2	0/3	0/3	2/3	1/2	0/2
	Perimysial fibrosis										
	very slight:	0/3	0/3	0/3	0/2	0/2	1/3	0/3	1/3	0/2	0/2
	slight:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Lungs	Bronchiolar alteration										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	0/3	2/2	1/2	0/3	0/3	0/3	0/2	1/2
	Pneumonia										
	very slight:	0/3	1/3	1/3	0/2	0/2	1/3	0/3	1/3	0/2	0/2
	slight:	2/3	0/3	0/3	0/2	0/2	2/3	2/3	0/3	0/2	0/2
	moderate:	1/3	1/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Edema										
	very slight:	0/3	1/3	0/3	0/2	0/2	0/3	1/3	0/3	0/2	0/2
	slight:	1/3	2/3	0/3	0/2	0/2	3/3	1/3	0/3	0/2	0/2
	moderate:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Macrophage infiltration										
	very slight:	0/3	0/3	1/3	0/2	1/2	0/3	0/3	1/3	0/2	0/2
	slight:	0/3	1/3	1/3	0/2	0/2	1/2	1/3	1/3	1/2	0/2
	moderate:	0/3	2/3	0/3	0/2	0/2	2/3	2/3	0/3	0/2	0/2
	Neutrophil infiltration										
	very slight:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	2/3	1/3	0/3	0/2	0/2	0/3	1/3	0/3	0/2	0/2
	Fibrin deposit										
	very slight:	0/3	1/3	0/3	0/2	0/2	0/3	1/3	0/3	0/2	0/2
	slight:	2/3	2/3	0/3	0/2	0/2	3/3	1/3	0/3	0/2	0/2
	moderate:	1/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Subpleural fibrosis										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	0/3	1/2	0/2	0/3	0/3	0/3	0/2	1/2
	moderate:	0/3	0/3	0/3	1/2	0/2	0/3	0/3	0/3	0/2	0/2
	Hyaline membrane										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	1/3	1/3	0/3	0/2	0/2	0/3	1/3	0/3	0/2	0/2
Liver	Brown Kupffer cell										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	slight:	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Hepatocyte necrosis										
	very slight:	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Cell infiltration, Glisson's										
	very slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	1/3	0/2	0/2
Spleen	White pulp atrophy										
	very slight:	0/3	0/3	0/3	2/2	1/2	1/3	1/3	0/3	2/2	1/2
	slight:	3/3	1/3	0/3	0/2	1/2	2/3	2/3	0/3	0/2	1/2
	moderate:	0/3	2/3	0/3	0/2	0/2	0/3	0/3	3/3	0/2	0/2

Tissue	Findings	MALE					FEMALE				
		Main			Rec.		Main			Rec.	
		3	10	30	10	30	3	10	30	10	30
Thymus	Atrophy of cortex										
	very slight:	2/3	2/3	0/3	1/2	1/2	2/3	0/3	3/3	0/2	2/2
	slight:	0/3	0/3	0/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	moderate:	0/3	0/3	3/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
	Enlarged medulla										
	very slight:	1/3	1/3	0/3	1/2	2/2	3/3	0/3	0/3	2/2	2/2
Mesenteric Lymph Node	Atrophy										
	very slight:	0/3	0/3	0/3	1/2	2/2	0/3	0/3	0/3	2/2	1/2
	slight:	3/3	2/3	0/3	1/2	0/2	3/3	3/3	3/3	0/2	1/2
	moderate:	0/3	1/3	3/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Submandibular Lymph Node	Atrophy										
	very slight:	0/3	0/3	0/3	1/2	2/2	0/3	0/3	0/3	2/2	1/2
	slight:	3/3	2/3	0/3	1/2	0/2	3/3	3/3	3/3	0/2	1/2
	moderate:	0/3	1/3	3/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Bone marrow	Decreased hematopoietic cells										
	very slight:	0/3	0/3	1/3	0/2	0/2	0/3	0/3	0/3	0/2	0/2
Testes	Hypospermatogenesis										
	very slight:	0/3	0/3	0/3	0/2	0/2	-	-	-	-	-
	slight:	0/3	0/3	2/3	0/2	0/2	-	-	-	-	-
	moderate:	0/3	0/3	1/3	0/2	0/2	-	-	-	-	-
Epididymides	Hypospermia										
	very slight:	0/3	0/3	0/3	0/2	0/2	-	-	-	-	-
	slight:	0/3	0/3	3/3	0/2	0/2	-	-	-	-	-

Peri. – perivascular, Rec. - recovery.

Toxicokinetics (Study №: DMPK-r6988)

Blood samples were taken from animals at the LD, MD and HD on Day 1 at pre-dose, 8 and 24 hours post-dose, in Weeks 2 and 4 of treatment at 8 and 24 hours and from MD and HD in Weeks 2 and 4 of recovery at 8 and 24 hours.

There was vomiting observed in 14 of 26 dogs after the first dose that may have affected the toxicokinetic parameters, especially in F. No general difference in plasma levels at 8 and 24 hours post-dose was observed between the sexes. Plasma levels increased dose-proportionally. Accumulation of 1.3-2.3-fold occurred between Days 14 and 28. There was still 6-12 ng/ml of FTY720 in 2 F recovery animals at 2 weeks, with none detected by Week 4 of the recovery period.

Table of male toxicokinetic parameters (Sponsor's)

Table 1 Unchanged drug concentrations In blood after repeated oral administration of Y-32720 to male beagles

Dose (mg/kg)	Animal No.	Blood concentration (ng/ml)							
		first day		14 days		28 days		14 days after	28 days after
		8 h	24 h	8 h	24 h	8 h	24 h		
3	2101	96.6	49.9	116.1	43.6	170.1	83.0	no sample	no sample
	2102	12.5	45.3	99.3	44.3	257.4	125.1	no sample	no sample
	2103	101.7	82.9	117.5	76.4	255.9	114.5	no sample	no sample
	mean	70.3	59.4	111.0	54.8	227.8	107.5		
	SD	50.1	20.5	10.1	18.7	50.0	21.9		
10	3101	210.8	101.1	504.4	206.8	590.2	232.0	no sample	no sample
	3102	316.5	217.7	524.4	307.9	1151.5	511.3	no sample	no sample
	3103	12.2	7.7	331.6	137.9	259.6	114.0	no sample	no sample
	3104	83.5	33.3	266.6	139.4	726.4	274.0	n.d.	n.d.
	3105	342.1	177.6	450.3	242.4	1193.9	479.8	n.d.	n.d.
	mean	193.0	107.5	415.5	206.9	784.3	322.2		
	SD	143.6	90.2	112.0	72.1	393.4	169.1		
30	4101	259.8	242.8	1632.1	1049.9	4811.8	2927.1	no sample	no sample
	4102	220.6	177.0	1801.0	1130.4	3012.1	1443.5	no sample	no sample
	4103	224.8	171.1	1997.5	1455.1	3717.3	1695.0	no sample	no sample
	4104	534.9	367.8	1189.6	635.4	2975.6	1069.1	n.d.	n.d.
	4105	746.3	341.6	314.2	305.2	1343.0	683.6	n.d.	n.d.
	mean	397.3	260.1	1386.9	915.2	3172.0	1563.7		
	SD	234.9	91.3	669.7	449.0	1264.5	852.9		

n.d. : below the limit of detection (<3 ng/ml).

Table of female toxicokinetic parameters (Sponsor's)

Table 2 Unchanged drug concentrations In blood after repeated oral administration of Y-32720 to female beagles

Dose (mg/kg)	Animal No.	Blood concentration (ng/ml)							
		first day		14 days		28 days		14 days after	28 days after
		8 h	24 h	8 h	24 h	8 h	24 h		
3	2201	73.2	64.7	188.8	105.0	386.0	159.6	no sample	no sample
	2202	103.0	57.2	160.0	95.1	399.7	154.3	no sample	no sample
	2203	83.3	64.0	156.9	90.8	262.0	153.5	no sample	no sample
	mean	86.5	62.0	168.6	97.0	349.2	155.8		
	SD	15.2	4.1	17.6	7.3	75.9	3.3		
10	3201	132.1	84.0	308.8	163.9	458.3	256.4	no sample	no sample
	3202	106.7	78.2	421.4	260.1	930.0	454.0	no sample	no sample
	3203	74.3	54.0	346.4	162.6	431.9	192.1	no sample	no sample
	3204	29.4	21.5	674.7	332.7	865.4	610.8	n.d.	n.d.
	3205	162.0	119.6	380.7	212.4	1273.6	1177.1	11.6	n.d.
	mean	100.9	71.5	426.4	226.3	791.8	538.1		
	SD	51.4	36.5	144.9	71.8	352.6	393.6		
30	4201	432.1	280.6	1312.5	780.7	751.1	429.8	no sample	no sample
	4202	935.3	762.1	2799.6	1840.1	2595.7	803.7	no sample	no sample
	4203	492.7	317.4	1739.4	1001.8	3587.0	469.0	no sample	no sample
	4204	501.6	244.5	1363.8	905.4	2523.1	1364.3	6.4	n.d.
	4205	1050.1	578.7	819.9	611.7	1072.9	2398.8	n.d.	n.d.
	mean	682.4	436.7	1607.0	1027.9	2106.0	1093.1		
	SD	287.4	224.5	742.5	476.9	1173.7	820.3		

n.d. : below the limit of detection (<3 ng/ml).

Stability and Homogeneity

There were no data presented.

Study title: Y-32720: Six-month oral toxicity study in dogs

Study no.:	PCS-r7393
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	16 December 1996
GLP compliance:	Yes (b) (4), 1982, 1988)
QA statement:	Yes (Dated: 28 Aug 1996, amended 11 Sep 1998)
Drug, lot #, and % purity:	Y-32720, Lot N, 100.1%

Key Study Findings

- Four animals administered 10 mg/kg/day died or were sacrificed early. Clinical signs observed in these animals included decreased locomotion, sedation, prone and lateral positions, labored breathing and hypothermia and they had decreased body weights and food consumption.
- White blood cells and lymphocytes were decreased and atrophy observed in lymphoid organs.
- Increased male and female lung weight and female heart weight was observed in animals dosed at 1 mg/kg/day and greater.
- Histopathological findings included brain mononuclear infiltration, peripheral nerve degeneration, heart fibrosis, lung macrophage infiltration, edema, pneumonia and congestion.
- Although myocardial fibrosis was observed at 0.01 mg/kg/day it was only 1 female and no other adverse findings were observed; therefore, the NOAEL is 0.01 mg/kg/day

Methods

Doses:	0, 0.001, 0.01, 1 and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	Gelatin capsules with 50 ml tap water
Formulation/Vehicle:	Lactate (30 mg/kg), apart from 10 mg/kg group
Species/Strain:	Dog/Beagle
Number/Sex/Group	
Main study:	4
Age:	8 months at start of treatment
Weight	
Males:	9.0-11.8 g
Females:	8.5-11.5 g

Observations and Results

Mortality

Four animals at the HD died or were sacrificed early; 2 M and 1 F were sacrificed in Weeks 7, 10 and 25, respectively, and 1 M was found dead in Week 23. Signs in these

animals included decreased locomotion, sedation, prone and lateral positions, labored breathing and hypothermia.

Clinical Signs
Observations were performed twice daily on weekdays, at pre-treatment and 3 times or more daily during treatment.

Loose stools and diarrhea were observed in control, MLD, MHD and HD groups; 1, 2 1 and 1 M at the MLD, MHD and HD, respectively, and 1, 2 and 2 F at control, MHD and HD, respectively. Salivation was observed in 2 M and 1 F at the HD and vomiting was observed in all dose groups.

Body Weights
Body weights were recorded pre-treatment and weekly during treatment.

There were no drug-related changes in body weight in animals at the LD, MLD and MHD. At the HD, body weight decreased from Day 18 in 1 M, with the dog sacrificed early in Week 7; 1 M had a decrease in body weight from Day 4 and was sacrificed early in Week 10. Of the remaining 2 M, 1 had a decrease in body weight on Day 151 and was found dead shortly after and the other survived to the end of the study, without loss of body weight. One F started to slowly lose body weight from Day 39 until early sacrifice in Week 25. The remaining F survived, but 2 had slight decreased body weight of 0.2 and 0.3 kg, compared to an increase in body weight of 0.4-1.1 kg in control.

Food Consumption
Food consumption was determined twice a week from pre-treatment until end of treatment.

There were no drug-related changes in food consumption in animals at the LD, MLD and MHD. At the HD, food consumption decreased in 1 M from Day 15 with the dog eating almost nothing about 1 week before sacrifice, early in Week 7. A second M had decreased food consumption from Day 1 on; food consumption slowly declined until about Day 53 and from then on consumed almost nothing until sacrificed in Week 10. The 2 remaining M had normal food consumption until 1 stopped eating about Day 149 and was found dead in Week 23. At the HD, F had mostly normal food consumption, although 3 had several days with a decreased consumption and the F sacrificed in Week 25 had consumed no food just before death.

Ophthalmoscopy and hearing
Examinations were performed 2 weeks pre-treatment and in Weeks 12 and 25.

No ophthalmoscopy data were submitted. It was reported that there were no ophthalmoscopic findings at the LD, MLD and MHD. At the HD in Week 12, 1 M and 1 F had vacuoles of the retina. At Week 25, the vacuoles had disappeared in the F; there were no data for the M, as it died in Week 23.

No auditory function data were submitted, it was reported that there was no effect on auditory functions.

Hematology
Blood samples were taken from all animals 1 week pre-treatment and in Weeks 4, 8, 16 and 26. The following parameters were assessed: erythrocyte, leukocyte and platelet counts, HGB, HCT and blood coagulation.

In M, RBC parameters (count, HGB and HCT) in HD animals that did not survive to the end of the study were slightly increased. In other HD M, at Week 26, WBC counts were decreased (compared to pre-treatment levels) in MLD and MHD by 40% and 17%, respectively; the individual M at the HD had a decrease of 60% (Week 4), 49% (Week 8), 47% (Week 16) and 5% (Week 26), before death. Lymphocyte counts were decreased by 87% and 94% in M at the MLD and MHD, respectively, with a decrease of 94% (Week 4), 97% (Week 8), 85% (Week 16) and 92% (Week 26) at the HD. Eosinophils were decreased at the MHD and in 1 HD M that died before the end of treatment.

In F at Week 16, WBC counts were decreased by 19%, 40%, 23% and 5% at the LD, MLD, MHD and HD, respectively, with lymphocytes decreased by 46%, 91%, 96% and 88%, respectively.

Clinical Chemistry
Blood samples were taken from all animals at 1 week pre-treatment and in weeks 4, 8, 16 and 26. The following parameters were assessed: GOT, GPT, ALP, CPK, total bilirubin, BUN, creatinine, calcium, phosphate, total cholesterol, phospholipids, triglycerides, glucose, total protein, sodium, potassium, chloride, albumin and globulins.

At Week 4 in M, a decrease from pre-treatment levels was observed in ALP, CPK, total cholesterol, phospholipids, total protein, glucose and an increase in α_1-globulin at the MHD and HD. In addition, α_2-globulin was increased at the HD.

In F, a decrease from pre-treatment levels was observed in ALP, total cholesterol, phospholipids, total protein and glucose and an increase was observed in α_2-globulin at the MHD and HD. In addition, α_1-globulin was increased at the HD.

Urinalysis
Urine samples were taken from all animals at 1 week pre-treatment and in Weeks 4, 8, 16 and 26.

Sodium was decreased at the LD, (18%), MLD (45%), MHD (54%) and HD in M, and pH was decreased in HD M.

Gross Pathology
Gross pathology was assessed in all animals.

Gross findings were mainly found at the HD in animals that died or were sacrificed early and included areas of dark red in the lungs, foamy mucus in the trachea and small thymuses.

Organ Weights

At necropsy, the following organs were weighed: brain, heart, lungs, liver, kidneys, spleen, hypophysis, adrenals, thyroid glands, pancreas, submandibular glands, thymus, prostate, ovary, uterus and testes.

In M, organ weights at the HD were collected from only the surviving animals, making comparisons with control animals difficult. In M, at the MHD, there was an increase in the relative weight of lungs (26%) and a decrease in kidney weight (11%). F organ weight changes included an increase at MHD and HD in lungs (21% and 79%), heart (11% and 14%) and a decrease at the HD in thymus (24%).

Histopathology

At necropsy, the following organs were prepared for histopathology examination: liver, kidneys, lungs, trachea, heart, spleen, pancreas, brain, hypophysis, spinal cord, sciatic nerve, adrenals, thyroid and parathyroid glands, thymus, submandibular and mesenteric lymph nodes, parotid, sublingual and submandibular glands, femur and sternum bone, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, gallbladder, aorta, prostate, ovary, uterus, vagina, mammary glands, skin, tongue and lacrimal glands. Tissues were fixed in 10% formalin, except for testes and epididymis that were fixed in Bouin's solution and eye balls that were fixed in formaldehyde-glutaraldehyde solution.

Adequate Battery: Yes
Peer Review: No

The lymphoid organs had findings of atrophy consistent with the pharmacological action of FTY720. Other findings included brain mononuclear infiltration, peripheral nerve degeneration, lacrimal acinar cell atrophy, heart fibrosis, lung macrophage infiltration, edema, pneumonia and congestion, kidney tubular atrophy, adrenal gland vacuolation and fibrosis and pituitary gland vacuolation.

Summary of major microscopic findings

		MALE					FEMALE				
Tissue	**Findings**	**0**	**0.001**	**0.01**	**1.0**	**10**	**0**	**0.001**	**0.01**	**1.0**	**10**
Brain	Mononuclear cell infiltration										
	very slight:	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**
	slight:	**0/4**	**0/4**	**0/4**	**0/4**	**1/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**
	Mononuclear cell aggregation										
	very slight:	**0/4**	**0/4**	**0/4**	**0/4**	**1/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**
	slight:	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**2/4**
Peripheral nerves	Heart nerve degeneration										
	very slight:	**0/4**	**0/4**	**0/4**	**0/4**	**2/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**
	slight:	**0/4**	**0/4**	**0/4**	**0/4**	**1/4**	**0/4**	**0/4**	**0/4**	**0/4**	**1/4**
	Auerbach plexus degeneration										
	very slight:	**0/4**	**0/4**	**0/4**	**0/4**	**3/4**	**0/4**	**0/4**	**0/4**	**0/4**	**1/4**

Tissue	Findings	MALE 0	MALE 0.001	MALE 0.01	MALE 1.0	MALE 10	FEMALE 0	FEMALE 0.001	FEMALE 0.01	FEMALE 1.0	FEMALE 10
Lacrimal gland	Acinar cell atrophy										
	very slight:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	1/4
Heart	Wall thickening										
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	2/4	2/4
	slight:	0/4	0/4	0/4	2/4	3/4	0/4	0/4	0/4	0/4	1/4
	Perivascular fibrosis										
	very slight:	0/4	0/4	0/4	1/4	2/4	0/4	0/4	0/4	0/4	1/4
	Perimysial fibrosis										
	very slight:	0/4	0/4	0/4	2/4	2/4	0/4	0/4	0/4	1/4	1/4
	Myocardial fibrosis										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4
Lungs	Macrophage infiltration										
	very slight:	0/4	1/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4	0/4
	slight:	0/4	0/4	0/4	2/4	1/4	0/4	0/4	0/4	1/4	2/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Neutrophil infiltration										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
	Edema										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4	0/4
	Subpleural fibrosis										
	very slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	Pneumonia										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
	Congestion										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	0/4
Liver	Glandular cell vacuolation										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Submandibular glands	Acinar cell atrophy										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
Kidneys	Tubular epithial atrophy										
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
Adrenal gland	Medullary cell vacuolation										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	2/4
	moderate:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Decreased medullary										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	2/4

Tissue	Findings	MALE 0	MALE 0.001	MALE 0.01	MALE 1.0	MALE 10	FEMALE 0	FEMALE 0.001	FEMALE 0.01	FEMALE 1.0	FEMALE 10
Adrenal gland	Medulla fibrosis										
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	2/4
Pituitary gland	Cyst										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	1/4	1/4	2/4	1/4	1/4	1/4	3/4	1/4	0/4	1/4
	Glandular cell vacuolation										
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
Spleen	Capsule thickening										
	very slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	White pulp atrophy										
	very slight:	0/4	0/4	3/4	0/4	0/4	0/4	0/4	2/4	1/4	0/4
	slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	3/4	0/4
	moderate:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	0/4	0/4	4/4	0/4	0/4	0/4	0/4	4/4
Thymus	Atrophy of cortex										
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	2/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	1/4
	Enlarged medulla										
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	2/4	0/4
Mesenteric Lymph Node	Atrophy										
	very slight:	0/4	0/4	3/4	1/4	0/4	0/4	0/4	3/4	1/4	0/4
	slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	1/4	3/4	3/4
	moderate:	0/4	0/4	0/4	1/4	4/4	0/4	0/4	0/4	0/4	1/4
Submandibular Lymph Node	Atrophy										
	very slight:	0/4	0/4	3/4	1/4	0/4	0/4	0/4	3/4	1/4	0/4
	slight:	0/4	0/4	0/4	2/4	1/4	0/4	0/4	1/4	3/4	3/4
	moderate:	0/4	0/4	0/4	1/4	3/4	0/4	0/4	0/4	0/4	1/4
Bone marrow	Decreased hematopoietic cell										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
Prostate	Epithelial cell atrophy										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
Testes	Hypospermatogenesis										
	very slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
Epididymides	Hypospermia										
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Special Evaluation

At necropsy, liver samples were taken from all dogs in the control, LD, LMD and HMD groups, microsomes and cytosol fractions were prepared and concentrations of cytochrome b_5 and P450 content determined and FTY720 hydroxylation activity measured.

Cytochrome b_5, P450 content was similar in all treatment groups assayed (not HD), with FTY720 also unchanged by treatment.

Toxicokinetics

Blood samples were taken on Day 1 and in Week 26 at 0.5, 1, 2, 4, 8, 12 and 24 hours for animals at the HD. Blood samples were taken in Week 26 at 8 and 24 hours for animals at the MHD. Blood samples were taken in Weeks 6, 13 and 18 at 8 and 24 hours for all animals. Brain samples were taken from the frontal lobe of all surviving dogs at necropsy and animals that died or were sacrificed early, №s 5103 and 5202.

Generally, there were no sex differences. At the HD, 24 hours post-dose plasma concentrations appeared approximately consistent from Week 13, although this cannot be certain for M at the HD, as 3 of the 4 dogs died before Week 26. Brain concentrations 24 hours post-last dose were generally dose-proportional with no gender differences.

Table of toxicokinetic parameters (Sponsor's)

At a time after first administration.

	5101	5102	5103	5104	Mean	SD		5201	5202	5203	5204	Mean	SD
0.5h				(b) (4)	34.5	17.9					(b) (4)	14.4	7.9
1 h					71.3	73.6						70.1	51.9
2 h					168.2	176.1						210.7	76.2
4 h					396.2	456.1						320.1	80.4
8 h					208.3	208.5						465.5	91.0
12 h					244.1	244.5						310.8	98.3
24 h					129.3	89.8						192.4	44.3

At 6 weeks after repeated administration.

	5101	5102	5103	5104	Mean	SD		5201	5202	5203	5204	Mean	SD
8 h				(b) (4)	1744.0	739.7					(b) (4)	1229.9	333.7
24 h					1037.7	420.1						769.1	163.5

At 13 weeks after repeated administration.

	5101	5102	5103	5104	Mean	SD		5201	5202	5203	5204	Mean	SD
8 h				(b) (4)	1213.5						(b) (4)	778.2	331.4
24 h					535.8							497.3	261.9

At 18 weeks after repeated administration.

	5101	5102	5103	5104	Mean	SD		5201	5202	5203	5204	Mean	SD
8 h				(b) (4)	856.1						(b) (4)	909.8	249.7
24 h					433.2							529.3	102.9

At 26 weeks after repeated administration.

	5101	5102	5103	5104	Mean	SD		5201	5202	5203	5204	Mean	SD
0.5h				(b) (4)							(b) (4)	555.4	74.7
1 h												595.0	108.7
2 h												651.7	100.4
4 h												763.1	162.0
8 h												812.8	139.3
12 h												688.0	114.1
24 h												439.2	26.6

No5101-5104 are male, and No5201-5204 are female beagles, respectively.
All data are shown as ng/ml blood.
The blood concentrations less than 10ng/ml(lower limit of quantitation) were shown as 10.

Stability and Homogeneity

Information was given that an assay was performed to confirm the stability of FTY720 in temperature, humidity and light.

Study title: Y32720: A 2-week oral toxicity study in Cynomolgus monkeys

Study no.:	PCS-r7102
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	23 March 1995
GLP compliance:	Yes ((b) (4), 1982)
QA statement:	Yes (Dated: 5 Sep 1995, amended 22 Oct 1996 and 24 Aug 1998)
Drug, lot #, and % purity:	Y32720, Lot N, 100.1%

Key Study Findings

- Slight decreases in body weights of 30 mg/kg/day animals occurred, accompanied by large decreases in food consumption.
- Decrease in lymphocytes and lymphoid atrophy at all doses was observed.
- Lung and heart weights were increased at all doses. Although there was a slight increase in lung weight at 3 mg/kg, there were few other signs of toxicity suggesting this was the NOAEL; with plasma exposure of 2,946 ng*h/ml.

Methods

Doses:	0, 3, 10 and 30 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group	
Main study:	2
Age:	3-7 years at start of treatment
Weight	
Males:	4.07-5.17 kg
Females:	2.48-3.46 kg

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

Observations were performed three times daily, pre-dose, immediately post-dose and 2-3 hours post-dose, during treatment.

Vomiting and reduced appetite were observed in animals at the HD. There were no drug-related clinical signs observed in animals at the LD and MD.

Body Weights
Body weights were recorded once pre-treatment and weekly during treatment.

There was a slight decrease in body weight in animals at the HD (5-7%).

Food Consumption
Food consumption was estimated once pre-treatment and weekly during treatment.

There was decrease in food consumption of 1 M (72%) and both F (42%) at the HD.

Ophthalmoscopy and hearing
Examinations of all animals were performed pre-treatment and on Day 11.

There were no treatment-related changes on ophthalmoscopy.

Blood pressure
Blood pressure was measured pre-dose and 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose on Days 0 and 12 of treatment.

There were no treatment-related changes in blood pressure.

Hematology
Blood samples were taken from all animals pre-treatment, on Day 1 and the day of necropsy. The following parameters, listed in the study report, were assessed.

Parameters	Methods	Apparatus
Erythrocyte count (RBC)	Detection system by electric resistance	E-4000 *
Leukocyte count (WBC)	Detection system by electric resistance	E-4000 *
Hematocrit value (Ht)	Detection by pulse method	E-4000 *
Hemoglobin concentration (Hb)	Method of sodium lauryl sulfate hemoglobin	E-4000 *
Blood platelet count (Plt)	Detection system by electric resistance	E-4000 *
Mean corpuscular volume (MCV)	Calculation	E-4000 *
Mean corpuscular hemoglobin (MCH)	Calculation	E-4000 *
Mean corpuscular hemoglobin concentration (MCHC)	Calculation	E-4000 *
Reticulocyte count (Ret)	New methylene blue · Wright double staining method	MICROX HEG-70 **
Differential leukocyte	Wright staining method	MICROX HEG-70 **
Prothrombin time (PT)		CA-5000 ***
Activated partial thromboplastin time (APTT)		CA-5000 ***

Multipurpose automatic cell counter (E-4000, Toa Medical Electronics)

Blood cell autoanalyzer (MICROX HEG-70, Omron, Co.)

* Automatic blood coagulation measuring apparatus (CA-5000, Toa Medical Electronics)

Reticulocytes, PT and APTT were not measured on Day 1 of the dosing.

There was a decrease in WBC counts and a 36-82% decrease in lymphocytes in animals treated at LD, MD and HD.

Clinical Chemistry

Blood samples were taken from all animals pre-treatment and the day of necropsy. The following parameters, listed in the study report, were assessed.

Parameter	Method	Apparatus
Aspartate aminotransferase (ASAT)	Modified JSCC method	RX-10 *
Alanine aminotransferase (ALAT)	Modified JSCC method	RX-10 *
Alkaline phosphatase (ALP)	p-nitrophenyl phosphoric acid substrate method	RX-10 *
Lactate dehydrogenase (LDH)	UV Rate assay	RX-10 *
Creatine phosphokinase (CPK)	UV Rate assay	RX-10 *
Total bilirubin	Alkaline azo bilirubin method	RX-10 *
Total protein	Biuret method	RX-10 *
Albumin	BCG method	RX-10 *
Total cholesterol	COD-DAOS method	RX-10 *
Triglyceride	GPO-DAOS method	RX-10 *
Glucose	GlcK · G-6-PDH method	RX-10 *
Phospholipid	Enzyme method	RX-10 *
Blood urea nitrogen	Urease-GlDH method	RX-10 *
Creatinine	Jaffé method	RX-10 *
Uric acid	Uricase · TOOS Method	RX-10 *
Inorganic phosphorus (IP)	Molybdic acid direct method	RX-10 *
Calcium (Ca)	OCPC method	RX-10 *
Sodium (Na)	Electrode method	RX-10 *
Potassium (K)	Electrode method	RX-10 *
Chloride (Cl)	Coulometric-titration method	RX-10 *
Protein fractions (including A/G ratio)	Electrophoresis method	FED-II *

* Clinalyzer (RX-10, JEOL, Ltd.)

** Automatic electrophoresis apparatus (MODEL FED-II, Cosmo Co., Ltd.)

There was an increase in BUN and creatinine in 1 M at the HD. Otherwise, there were no changes.

Urinalysis

Samples (fresh, 2 hour and preserved, 18 hour) were taken from all animals pre-treatment and on Day 9 of treatment. The following parameters, listed in the study report, were assessed.

Parameter	Method	Apparatus
Fresh Urine (2-hour sample)		
Color	Visual	
pH	Test paper	Clinitek 200+ *
Protein	Test paper	Clinitek 200+ *
Glucose	Test paper	Clinitek 200+ *
Ketone body	Test paper	Clinitek 200+ *
Bilirubin	Test paper	Clinitek 200+ *
Urine occult blood	Test paper	Clinitek 200+ *
Urobilinogen	Test paper	Clinitek 200+ *
Urine sediments	Microscopic examination of urine sediments stained with Sternheimer-Malbin stain after centrifugation (1500 r.p.m., 5 minutes).	
18-hour Excreted Urine		
Urine volume	Measuring cylinder	
Urine specific gravity	Urinary refractometer	URICON-S **
Sodium	Electrode method	IT-3 ***
Potassium	Electrode method	IT-3 ***
Chloride	Coulometric titration method	IT-3 ***

* Automatic urine analyzer (Clinitek 200+, Miles Labs., Inc., U S A)

** Urinary refractometer (URICON-S, Atago Co., Ltd.)

*** Automatic electrolyte analyzer (IT-3, Jookoo, Co., Ltd.)

Total excretion volume of the urinary electrolytes was calculated from urine volume and electrolyte concentration.

There were no drug-related changes in urinalysis parameters.

Gross Pathology

Gross pathology was assessed in all animals.

Subendocardial hemorrhage of the left ventricle was seen in 1 M heart at the LD and small cyst in the kidney of 1 M at the HD. Otherwise, no macroscopic changes were observed.

Organ Weights

At necropsy, the following organs were weighed: brain, pituitary, thyroids, submandibular glands, thymus, heart, lungs, liver, adrenals, kidneys, spleen, pancreas, testes, epididymides, seminal vesicle, prostate, ovary and uterus.

In M, there was an increase in the relative weights of lungs (15%) at the LD. At the MD, heart (16%) and lung (32%) weights were increased. At the HD, brain (17%), heart (12%) and lung (46%) weights were increased and pancreas (28%), seminal vesicle (48%) and prostate (34%) weights were decreased.

In F, there were decreases in relative weights of the pituitary (32%), thyroid (38%), adrenal (24%), pancreas (25%) and kidneys (22%) at the HD.

Histopathology

At necropsy, the following organs were prepared for histopathology examination: heart, aorta, spleen, thymus, bone marrow, bone, submandibular and mesenteric lymph nodes, lungs, bronchus, trachea, tongue, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (cecum, colon and rectum), pancreas, liver, gall bladder, kidneys, urinary bladder, seminal vesicle, prostate, ovary, vagina, pituitary, thyroid, parathyroid and adrenal glands, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, skeletal muscle, mammary gland, skin, submandibular glands. Tissues were fixed in 10% formalin, except for testes and epididymides that were fixed in Bouin's solution and eye balls and lacrimal gland that were fixed in formaldehyde-glutaraldehyde solution.

Adequate Battery: Yes
Peer Review: No

The lymphoid organs had findings of atrophy consistent with the pharmacological action of FTY720. Other findings included lung smooth muscle hyperplasia in 1 of 4 animals at the HD, heart necrosis in 1 of 4 animals at the HD and histiocyte accumulation in the GI tract in animals at the MD and HD.

Summary Table of major microscopic findings with FTY720

		MALE				FEMALE			
Tissue	**Finding**	**0**	**3**	**10**	**30**	**0**	**3**	**10**	**30**
Heart	Left ventricular hemorrhage								
	very slight:	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**
	slight:	**0/2**	**1/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**
	Mononuclear cell infiltration								
	very slight:	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**1/2**	**0/2**	**0/2**
	Necrosis								
	very slight:	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**
	slight:	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**1/2**
Lungs	Smooth muscle hyperplasia								
	very slight:	**0/2**	**0/2**	**0/2**	**1/2**	**0/2**	**0/2**	**0/2**	**0/2**
Kidneys	Tubular atrophy								
	very slight:	**0/2**	**0/2**	**1/2**	**0/2**	**0/2**	**0/2**	**0/2**	**1/2**
	slight:	**0/2**	**0/2**	**0/2**	**1/2**	**0/2**	**2/2**	**0/2**	**1/2**
	Cyst								
	very slight:	**0/2**	**1/2**	**0/2**	**0/2**	**1/2**	**2/2**	**0/2**	**0/2**
	slight:	**0/2**	**0/2**	**0/2**	**1/2**	**1/2**	**1/2**	**0/2**	**0/2**
	Mononuclear cell infiltration								
	very slight:	**0/2**	**0/2**	**0/2**	**1/2**	**0/2**	**0/2**	**1/2**	**1/2**
	slight:	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**2/2**	**0/2**	**0/2**
	Mineralization								
	very slight:	**1/2**	**0/2**	**1/2**	**0/2**	**1/2**	**0/2**	**1/2**	**1/2**
	slight:	**0/2**	**1/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**

Tissue	Finding	MALE 0	MALE 3	MALE 10	MALE 30	FEMALE 0	FEMALE 3	FEMALE 10	FEMALE 30
Stomach	Fundus dilatation								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	slight:	0/2	0/2	1/2	0/2	0/2	0/2	0/2	1/2
	moderate:	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
Duodenum	Histiocyte accumulation								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2
	slight:	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
Jejunum	Histiocyte accumulation								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	slight:	0/2	0/2	1/2	0/2	0/2	0/2	0/2	1/2
	moderate:	0/2	0/2	0/2	2/2	0/2	0/2	0/2	1/2
Ileum	Histiocyte accumulation								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	slight:	0/2	0/2	1/2	1/2	0/2	0/2	0/2	1/2
	moderate:	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
Spleen	Atrophy								
	very slight:	0/2	2/2	1/2	1/2	1/2	2/2	2/2	0/2
	slight:	0/2	0/2	1/2	1/2	0/2	0/2	0/2	2/2
	Brown pigment in red pulp								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	slight:	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
Thymus	Atrophy								
	very slight:	0/2	1/2	1/2	1/2	1/2	0/2	0/2	1/2
	slight:	1/2	0/2	0/2	0/2	1/2	0/2	0/2	1/2
	moderate:	1/2	0/2	1/2	1/2	0/2	1/2	0/2	0/2
	Cyst								
	very slight:	0/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
Mesenteric Lymph Node	Histiocytosis								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	slight:	0/2	0/2	1/2	0/2	0/2	0/2	0/2	1/2
	moderate:	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
Ovary	Oocyte mineralization								
	very slight:	0/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2
	Slight:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	moderate:	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2

Special Evaluation

Hormones: Blood samples were taken pre-treatment, in Week 1 and at necropsy for the analysis of T3, T4, TSH, ACTH and adrenalin.

There were no treatment-related changes in hormone levels.

Toxicokinetics (Study №: DMPK-r7456)

Blood samples were taken on Day 1 and last day, at pre-dose and 0.5, 1, 2, 4, 8, 12 and 24 hours. Blood samples were also taken on Day 6, at 8 and 24 hours post-dose.

Generally, there were no sex differences; plasma exposure was approximately dose-proportional with accumulation over the course of the study. $AUC_{0\text{-}24h}$ for M at the HD were 14,019 and 39,206 ng*h/ml and for F at the HD were 12,835 and

32,849 ng*h/ml at Day 1 and Week 2, respectively. T_{max} values ranged between 2-18 hours. Brain to blood and lung to blood ratios 24 hours post-last dose were 105-154 and 185-241, respectively.

Table of toxicokinetic parameters

	Day 1			Last day (14)		
mg/kg/day	3	10	30	3	10	30
Males						
T_{max} (h)	4	6	18	6	6	5
C_{max} (ng/ml)	84.15	364.75	949.05	166.65	725.85	2037.4
$AUC_{(0\text{-}24\ h)}$ (ng*h/ml)	1,350.2	5,675.1	14,018.7	2,985.6	12,753.45	39,206.05
Accumulation				2.21	2.25	2.80
Females						
T_{max} (h)	4	3	6	2	4	7
C_{max} (ng/ml)	75.1	303.5	833.2	193.65	565.1	1750.4
$AUC_{(0\text{-}24\ h)}$ (ng*h/ml)	1,217.2	4,016.85	12,834.75	2,906.1	7,891.35	32,848.55
Accumulation				2.39	1.96	2.56
Both						
$AUC_{(0\text{-}24\ h)}$ (ng*h/ml)	1,283.7	4,846.0	13,426.7	2,945.8	10,322.4	36,027.3
Accumulation				2.29	2.13	2.68

Table of FTY720 concentrations in brain, lung and blood 24 hours post-last dose (Sponsor's)

Dose (mg/kg)	Animal No.	Sex	Concentration		
			Brain (μg/g)	Lung (μg/g)	Blood$_{24h}$ (μg/ml)
3	5	Male	13.26(161)[a]	15.87(192)[b]	0.08(1)
	6	Male	13.64(146)	25.08(269)	0.09(1)
	7	Female	10.15(171)	16.01(270)	0.06(1)
	8	Female	13.65(139)	23.09(235)	0.10(1)
		Mean	12.68(154)	20.01(241)	0.08(1)
		SD	1.69	4.77	0.02
10	9	Male	33.57(133)	32.95(131)	0.25(1)
	10	Male	49.56(93)	81.91(154)	0.53(1)
	11	Female	32.94(146)	63.38(282)	0.23(1)
	12	Female	24.83(159)	28.46(182)	0.16(1)
		Mean	35.23(133)	51.67(187)	0.29(1)
		SD	10.35	25.43	0.17
30	13	Male	47.32(123)	75.77(197)	0.39(1)
	14	Male	189.71(76)	389.35(157)	2.48(1)
	15	Female	115.09(123)	184.27(198)	0.93(1)
	16	Female	84.40(98)	161.99(188)	0.86(1)
		Mean	109.13(105)	202.85(185)	1.17(1)
		SD	60.44	132.85	0.91

( ):Values in parenthesis indicate relative concentrations based

Stability and Homogeneity
No data were submitted.

Study title: Y32720: A 13-week oral toxicity study in Cynomolgus monkeys

Study no.:	PCS-r7471
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 December 1995
GLP compliance:	Yes (b) (4) 1982)
QA statement:	Yes (Dated: 25 Oct 1996 and 24 Aug 1998)
Drug, lot #, and % purity:	Y32720, Lot N, 100.1%

Key Study Findings

- Decrease in lymphocytes and findings of atrophy in lymphoid organs at all doses.
- Several organs increased in weight, including lungs and heart.
- At 1 mg/kg/day lung weight was increased and lung smooth muscle hypertrophy was observed; therefore a NOAEL was not established.

Methods

Doses:	0, 1, 3 and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group	
Main study:	3
Recovery 8 weeks:	2 for control, MD and HD groups
Age:	3-5 years at start of treatment
Weight	
Males:	3.32-5.19 kg
Females:	2.71-3.78 kg

Observations and Results

Mortality
There were no deaths during the study.

Clinical Signs
Observations were performed once daily pre-treatment and recovery and at least 3 times daily during treatment; pre-dose, 0-1 and 2-3 hours post-dose.

Vomiting and loose stools were observed in animals at the HD. There were no drug-related clinical signs observed in animals at the LD and MD.

Body Weights
Body weights were recorded once pre-treatment and then weekly during treatment and recovery.

There was a slight decrease at the HD, in M and F body weight gain, by 40% (238 g) and 23% (24 g), respectively.

Food Consumption
Food consumption was estimated daily from 7 days pre-treatment until 7 days after treatment initiation and then weekly until end of recovery.

At the HD, there was a slight decrease in food consumption that was observed from Week 1 in 2 M and Week 2 in another M. In F, there was decrease in food consumption in all groups during Weeks 1-3; 12% in 2 at the LD, 24% in 2 at the MD and 44% in 3 at the HD, compared to control.

Ophthalmoscopy
Examinations of all animals were performed pre-treatment and at Weeks 12.

There were no treatment-related changes on ophthalmoscopy.

Blood pressure and ECG
Blood pressure was measured pre-dose and 4 hours post-dose at Weeks 4 and 13 of treatment.

There were no drug-related changes in blood pressure. However, the raw data were labeled as pre and 24 hour time points, not 4 hour. Furthermore, no control data were submitted.

Hematology
Blood samples were taken from all animals pre-treatment and in Week 13 of treatment and Week 8 of recovery. The following parameters, listed in the study report, were assessed.

- erythrocyte count (RBC : electric resistance detection method)
- leukocyte count (WBC : electric resistance detection method)
- platelet count (electric resistance detection method)
- hematocrit value (cumulative pulse height detection method)
- hemoglobin concentration (sodium lauryl sulfate hemoglobin method)
- mean corpuscular volume (MCV)
- mean corpuscular hemoglobin (MCH)
- mean corpuscular hemoglobin concentration (MCHC)

In M, decreased WBC counts was observed in all treatment groups, 24 hours post-first dose (1.3-35.4%), counts decreased further to 42-60.5% of pre-treatment levels by Week 13. There was some reversal by Week 8 of recovery. Lymphocyte count was decreased (61.4-68.5%) after 24 hours and were still decreased (62.5-77.3%) in Week 13. In addition, there was a decrease in PT.

In F, decreased WBC counts were observed at all doses (37.7-57.5% of pre-treatment levels at Week 13). There was some reversal by Week 8 of recovery. Lymphocyte counts, at all doses, were decreased by 68.1-72.2% at Week 13. In addition, neutrophils increased by 50.5-60.8% at Week 13.

Clinical Chemistry

Blood samples were taken from all animals pre-treatment and in Week 13 of treatment and Week 8 of recovery.

At the HD, M had increased levels in ALAT (81%), total cholesterol (30%), phospholipids (32%), and β-globulin (32%) that resulted in decrease of A/G ratio. Changes in F at the HD included increased CPK (137%), phospholipids (10%) and β-globulin (25%) that resulted in decrease of A/G ratio and a decrease in total protein (14%). There was no reversal in the changes of ALAT in M or in β-globulin in M and F.

Urinalysis

Samples (fresh, 2 hour and preserved, 18 hour) were taken from all animals pre-treatment and in Week 13 of treatment and Week 8 of recovery.

There were no drug-related changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

Subendocardial hemorrhage was observed in animals at the MD and HD occurring in 1 right ventricle at the MD, in 3 and 4 left ventricles at the MD and HD, respectively, and 1 and 3 papillary muscles at the MD and HD, respectively.

Organ Weights

At necropsy, the following organs, listed in the study report, were weighed.

- brain (with cerebellum and brain stem)
- thyroids (R&L, with parathyroids)
- submandibular glands (R&L)
- lung (with bronchus)
- adrenals (R&L)
- spleen
- testes (R&L)
- seminal vesicle
- ovaries (R&L)
- pituitary
- thymus
- heart
- liver
- kidneys (R&L)
- pancreas
- epididymides (R&L)
- prostate
- uterus

The thyroids were weighed after fixation with formalin.

In M, there was an increase in the relative weights of the thyroid glands (44%, 57% and 48%), lungs (27%, 28% and 43%), kidneys (3%, 7% and 16%), seminal vesicles (255%, 87% and 177%) at the LD, MD and HD, respectively, heart (19% and 19%) at MD and HD, respectively, and liver (19%) at the HD, and a decrease in thymus (22%, 24% and 65%) and spleen (50%, 51% and 51%) weights at the LD, MD and HD, respectively. The thyroids, thymus, spleen and seminal vesicles weights showed recovery, but heart and lung weights were still slightly increased in recovery animals, and kidneys increased further at the MD and HD to 9% and 20% of control values, respectively.

In F, increased relative weight was observed in thyroid glands (89%, 22% and 48%), lungs (8%, 21% and 30%) and heart (24%, 12% and 28%) at the LD, MD and HD, respectively, and liver (19%) at the HD and decrease in relative weight of thymus (35%) at the HD and spleen (21% and 27%) at the MD and HD. The thyroid showed recovery; however, there was little recovery of relative spleen weight at the MD and HD (still decreased by16% and 28%, respectively), heart weight was still increased by 9% and 20%, respectively, and lungs still increased by 13% and 20%, respectively.

Histopathology

At necropsy, the following organs were prepared for histopathology examination: heart, aorta, spleen, thymus, bone marrow, bone, submandibular and mesenteric lymph nodes, lungs, bronchi, trachea, tongue, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (cecum, colon and rectum), pancreas, liver, gall bladder, kidneys, urinary bladder, seminal vesicle, prostate, ovary, uterus, vagina, pituitary, thyroid, parathyroid and adrenal glands, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, skeletal muscle, mammary gland, skin, submandibular glands. Tissues were fixed in 10% formalin, except for testes and epididymides that were fixed in Bouin's solution and eye balls, optic nerve and lacrimal gland that were fixed in formaldehyde-glutaraldehyde solution.

Adequate Battery: Yes
Peer Review: No

The lymphoid organs had findings of atrophy and lymphocyte depletion consistent with the pharmacological action of FTY720. Increased smooth muscle hypertrophy was the main finding in lungs and was observed in all treatment groups, but not in recovery animals. Heart findings included subendocardial hemorrhage and myocardium fibrosis.

Summary Table of major microscopic findings

		MALE							FEMALE						
		Main				Recovery			Main				Recovery		
Tissue	**Findings**	**0**	**1**	**3**	**10**	**0**	**3**	**10**	**0**	**1**	**3**	**10**	**0**	**3**	**10**
Cerebrum	Brown pigment														
	very slight:	**1/3**	**1/3**	**0/3**	**0/3**	**1/2**	**1/2**	**0/2**	**2/3**	**2/3**	**0/3**	**1/3**	**0/2**	**0/2**	**0/2**
	slight:	**0/3**	**0/3**	**1/3**	**0/3**	**0/2**	**0/2**	**0/2**	**0/3**	**0/3**	**0/3**	**0/3**	**0/2**	**0/2**	**0/2**
	Mononuclear cell infiltration														
	very slight:	**2/3**	**0/3**	**0/3**	**0/3**	**0/2**	**0/2**	**0/2**	**0/3**	**1/3**	**0/3**	**0/3**	**0/2**	**0/2**	**0/2**
	slight:	**0/3**	**0/3**	**1/3**	**0/3**	**0/2**	**0/2**	**0/2**	**0/3**	**0/3**	**0/3**	**0/3**	**0/2**	**0/2**	**0/2**

		MALE							FEMALE						
		Main				Recovery			Main				Recovery		
Tissue	Findings	0	1	3	10	0	3	10	0	1	3	10	0	3	10
Cerebellum	Brown pigment														
	very slight:	0/3	0/3	1/3	1/3	0/2	0/2	1/2	1/3	1/3	1/3	0/3	1/2	1/2	0/2
	Mononuclear cell infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Sciatic nerve	Mononuclear cell infiltration														
	very slight:	0/3	1/3	0/3	0/3	0/2	1/2	0/2	1/3	2/3	0/3	1/3	1/2	0/2	0/2
	Perimeurium fibrosis														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	1/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Heart	Subendocard. Hemorrhage														
	very slight:	0/3	1/3	1/3	0/3	0/2	0/2	0/2	0/3	0/3	2/3	2/3	0/2	0/2	0/2
	slight:	0/3	0/3	1/3	2/3	0/2	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2	0/2
	Mononuclear cell infiltration														
	very slight:	1/3	2/3	3/3	2/3	0/2	2/2	1/2	3/3	2/3	3/3	3/3	0/2	0/2	0/2
	slight:	1/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Myocardial fiber necrosis														
	very slight:	1/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	1/2
	Myocardial fiber vacuolation														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2	0/2
	slight:	1/3	1/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Myocardium fibrosis														
	very slight:	0/3	1/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Myocardium hemorrhage														
	very slight:	0/3	1/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Aorta	Intimal thickening														
	very slight:	0/3	1/3	0/3	1/3	1/2	2/2	2/2	2/3	1/3	2/3	2/3	1/2	0/2	1/2
Lungs	Alveolar septa thickening														
	very slight:	0/3	1/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Pleura thickening														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	1/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Foamy cells														
	very slight:	0/3	1/3	1/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Granulation														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	moderate:	0/3	0/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Smooth muscle hypertrophy														
	very slight:	1/3	1/3	1/3	2/3	0/2	0/2	0/2	0/3	2/3	3/3	1/3	0/2	0/2	0/2
	slight:	0/3	2/3	2/3	0/3	0/2	0/2	0/2	0/3	1/3	0/3	1/3	0/2	0/2	0/2
	moderate:	0/3	0/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Microgranuloma														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	1/3	0/3	1/3	0/2	0/2	0/2
Bronchus	Bronchiole dilatation														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	moderate:	0/3	0/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Eosinophil infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	1/2	1/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	1/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Kidneys	Glomerulosclerosis														
	very slight:	1/3	0/3	2/3	0/3	0/2	1/2	1/2	3/3	0/3	1/3	2/3	0/2	1/2	1/2

		MALE							FEMALE						
		Main				Recovery			Main				Recovery		
Tissue	Findings	0	1	3	10	0	3	10	0	1	3	10	0	3	10
Kidneys	Tubular regeneration														
	very slight:	0/3	0/3	1/3	0/3	0/2	0/2	1/2	1/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	1/2
	Tubular hemorrhage														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2	0/2
	Mononuclear cell infiltration														
	very slight:	2/3	2/3	3/3	2/3	1/2	1/2	2/2	3/3	3/3	2/3	3/3	2/2	1/2	1/2
	Mineralization														
	very slight:	0/3	2/3	2/3	2/3	0/2	1/2	2/2	3/3	2/3	1/3	1/3	0/2	1/2	2/2
	Brown pigment														
	very slight:	0/3	0/3	0/3	1/3	1/2	0/2	1/2	0/3	0/3	0/3	0/3	0/2	2/2	1/2
	Hyaline cast														
	very slight:	0/3	0/3	0/3	0/3	0/2	1/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2	1/2
Trachea	Mononuclear cell infiltration														
	very slight:	0/3	1/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Eosinophil infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	1/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Granulation														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2	0/2
Stomach	Fundus dilatation														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	1/3	0/2	1/2	0/2
	Inflammation cell infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	1/3	0/3	0/3	0/2	0/2	0/2
Jejunum	Granuloma														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Cecum	Brown pigment														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	1/3	0/3	1/2	0/2	0/2
	slight:	0/3	0/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
Liver	Necrosis														
	very slight:	0/3	1/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Microgranuloma														
	very slight:	0/3	1/3	0/3	1/3	0/2	0/2	0/2	2/3	0/3	1/3	0/3	1/2	1/2	1/2
	Mononuclear cell infiltration														
	very slight:	2/3	1/3	1/3	0/3	1/2	1/2	1/2	3/3	1/3	2/3	2/3	0/2	1/2	0/2
Spleen	Atrophy														
	very slight:	0/3	0/3	0/3	2/3	0/2	0/2	0/2	0/3	1/3	3/3	1/3	1/2	0/2	0/2
	slight:	0/3	1/3	1/3	1/3	0/2	0/2	0/2	0/3	1/3	0/3	2/3	0/2	0/2	0/2
	moderate:	0/3	0/3	1/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Brown pigment in red pulp														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	1/2	0/2
Thymus	Atrophy														
	very slight:	0/3	0/3	1/3	2/3	1/2	1/2	1/2	1/3	0/3	1/3	1/3	0/2	2/2	0/2
	slight:	0/3	1/3	1/3	0/3	0/2	0/2	0/2	1/3	1/3	0/3	0/3	0/2	0/2	0/2
	Cyst														
	very slight:	0/3	2/3	0/3	1/3	2/2	0/2	1/2	0/3	0/3	0/3	0/3	0/2	2/2	0/2
Mesenteric Lymph Node	Atrophy														
	very slight:	0/3	1/3	1/3	1/3	0/2	0/2	0/2	1/3	2/3	3/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	1/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2

		MALE							FEMALE						
		Main				Recovery			Main				Recovery		
Tissue	Findings	0	1	3	10	0	3	10	0	1	3	10	0	3	10
Mesenteric Lymph Node	Eosinophil infiltration														
	very slight:	2/3	1/3	1/3	1/3	0/2	2/2	0/2	0/3	1/3	0/3	1/3	0/2	0/2	0/2
	slight:	0/3	1/3	0/3	1/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	0/2	0/2
	Brown pigment														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	0/2	1/2	0/2
	Macrophage infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	1/2	2/2	0/2
Submandibular Lymph Node	Atrophy														
	very slight:	0/3	2/3	1/3	3/3	0/2	0/2	0/2	0/3	2/3	2/3	2/3	0/2	0/2	0/2
	slight:	0/3	0/3	1/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	1/3	0/2	0/2	0/2
	Eosinophil infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	0/3	1/2	0/2	2/2
Adrenal gland	Brown pigment														
	very slight:	0/3	1/3	0/3	1/3	0/2	0/2	0/2	0/3	2/3	0/3	0/3	0/2	0/2	0/2
	Mononuclear cell infiltration														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	0/3	1/3	0/2	1/2	1/2
	Mineralization														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	1/2	0/3	1/3	0/3	0/3	0/2	0/2	0/2
	slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	0/3	1/3	0/3	0/2	0/2	0/2
	Hypertrophy														
	very slight:	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/3	1/3	1/3	0/3	0/2	0/2	0/2
Prostate	Mononuclear cell infiltration														
	very slight:	1/3	2/3	2/3	2/3	0/2	1/2	1/2	-	-	-	-	-	-	-
	Mineralization														
	very slight:	0/3	0/3	0/3	0/3	0/2	1/2	0/2	-	-	-	-	-	-	-
Ovary	Oocyte mineralization														
	very slight:	-	-	-	-	-	-	-	2/3	1/3	2/3	1/3	0/2	2/2	2/2
	slight:	-	-	-	-	-	-	-	0/3	1/3	1/3	0/3	0/2	0/2	0/2

Blood hormone

Blood samples were collected pre-treatment and at Weeks 5 and 10 during treatment. Serum T3, T4, TSH, prolactin, cortisol, aldosterone, adrenaline, noradrenaline and ACTH were measured.

There was no treatment effect on T4, TSH, prolactin, cortisol, aldosterone, adrenaline, noradrenaline or ACTH levels. T3 was decreased in M at the HD in Week 5 and F at the MD and HD in Weeks 5 and 10.

Electron microscopy

Liver, kidneys, cerebrum, brain stem and spinal cord from 2 animals from each group were fixed with 2% glutaraldehyde and 1% osmium tetraoxide and embedded in epoxy resin for electron microscopy.

There was a slight increase in cytoplasmic glia filaments of astrocytes in the cerebral gray matter from 1 M at the HD.

Toxicokinetics (Study №: DMPK-r7456)
Blood samples were taken on Day 0 at pre-dose and 1, 4, 8 and 24 hours post-dose, in Week 6 at 8 and 24 hours post-dose and on the last day at pre-dose and 1, 4, 8, 24, 48, 72 and 96 hours post-dose.

Generally, there were no sex differences; however, plasma exposure was greater in M than F at the HD. Plasma exposure was approximately dose-proportional with accumulation over the course of the study; $AUC_{0\text{-}24h}$'s at the HD were 11,463 and 15,688 ng*h/ml for M and 4,450 and 9,335 ng*h/ml for F at Day 1 and Week 13, respectively. T_{max} values were 5.3-8 hours.

Table of toxicokinetic parameters

	Day 1			Week 13		
mg/kg/day	1	3	10	1	3	10
Males						
T_{max} (h)	6.7	7.2	6.4	5.3	7.2	8
C_{max} (ng/ml)	20.7	69.9	722.7	54.5	127.9	820.9
$AUC_{(0\text{-}24\ h)}$ (ng*h/ml)	382	1,209.0	11,463.4	638.9	2,488.1	15,687.6
Accumulation		3.2	9.5	1.7	2.1	1.4
Females						
T_{max} (h)	8	7.2	6.4	6.7	6.4	5.6
C_{max} (ng/ml)	22.5	86.1	263.6	32.2	102.0	526.8
$AUC_{(0\text{-}24\ h)}$ (ng*h/ml)	418.9	1,519.8	4,450.1	655.3	1,910.4	9,335.3
Accumulation		3.6	2.9	1.6	1.3	2.1

Table of FTY720 concentrations in brain and blood (Sponsor's)

Dose (mg/kg)	Concentration (μg/g or μg/ml)					
	Animal	Male		Animal	Female	
	No.	Brain	$Blood_{24h}$	No.	Brain	$Blood_{24h}$
1	11	15.77(298) a)	0.05(1)	14	7.35(356) a)	0.02(1)
	12	8.32(316)	0.03(1)	15	6.19(366)	0.02(1)
	13	8.06(424)	0.02(1)	16	6.47(300)	0.02(1)
	Mean	10.71(346)	0.03(1)	Mean	6.67(341)	0.02(1)
	SD	4.38	0.02	SD	0.60	0.00
3	19	16.49(190)	0.09(1)	24	6.72(117)	0.06(1)
	20	12.29(125)	0.10(1)	25	7.37(120)	0.06(1)
	21	13.70(141)	0.10(1)	26	7.32(105)	0.07(1)
	Mean	14.16(152)	0.09(1)	Mean	7.14(114)	0.06(1)
	SD	2.14	0.01	SD	0.36	0.01
10	29	82.49(120)	0.69(1)	34	27.67(169)	0.16(1)
	30	36.35(85)	0.43(1)	35	22.04(159)	0.14(1)
	31	36.23(145)	0.25(1)	36	82.51(244)	0.34(1)
	Mean	51.69(117)	0.46(1)	Mean	44.07(191)	0.21(1)
	SD	26.67	0.22	SD	33.41	0.11

a) : C_{brain}/C_{blood}

Stability and Homogeneity
Not performed.

Study title: 39-Week oral (gavage) toxicity study in monkeys with a 13-week and 26-week recovery period

Study no.:	PCS-r997040-02
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	Novartis Pharmaceutical Corporation, East Hanover, New Jersey USA
Date of study initiation:	1 March 1999
GLP compliance:	Yes (Switzerland, 1986)
QA statement:	Yes (Dated: 24 Apr 2002, amended 29 Aug 2006)
Drug, lot #, and % purity:	FTY720, Batches 98905 and 9922007, 100%

Key Study Findings

- Decreased heart rate was observed up to Week 26 in males and Week 12 in females, QTc was increased in males up to Week 26. In addition, tall t-waves were observed in several animals at 3 mg/kg/day.
- Macroscopic findings in the lungs included red foci and lungs not collapsed that were also observed in animals after 13 weeks recovery. Lung microscopic findings included distended alveolus that only partial recovered after 13 weeks, increased collagen and smooth muscle hypertrophy that only partially recovered after 26 weeks.
- Due to the lung findings a NOAEL was not established.

Methods

Doses:	0, 0.5 and 3 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group	
Main study:	4
Recovery 13 weeks	3
Recovery 26 weeks	3
Age:	3-4 years at start of treatment
Weight	
Males:	2.6-4.3 kg
Females:	2.4-3.5 kg

Observations and Results

Mortality
Observations were performed twice daily (am and pm) on weekdays and daily at weekends and on holidays.

At the HD, 1 M was found dead on Day 130, but displayed no clinical signs. The Sponsor stated that the death was an accident and not treatment related.

Clinical Signs
Observations were performed at least once daily pre-treatment and recovery and at least twice daily during treatment; pre-dose and approximately 2 hours post-dose.

There were no drug-related clinical signs observed during the study.

Body Weights
Body weights were recorded weekly prior to treatment, during treatment and recovery.

There were no drug-related changes in body weight over the course of treatment.

Food Consumption
Food consumption was estimated daily from pre-treatment until end of recovery.

There were no treatment-related changes in food consumption over the course of treatment with decreases observed occasionally in all groups.

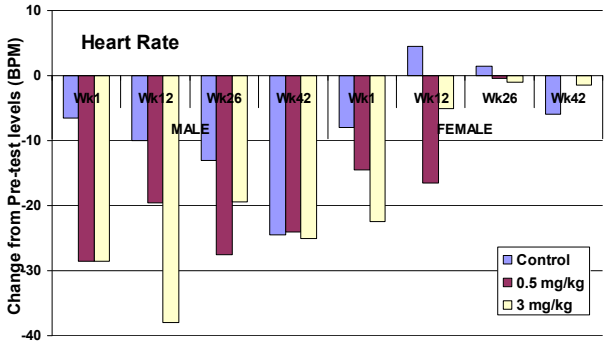
Ophthalmoscopy and hearing
Examinations of all animals were performed pre-treatment and during Weeks 4, 12, 27 and 43.

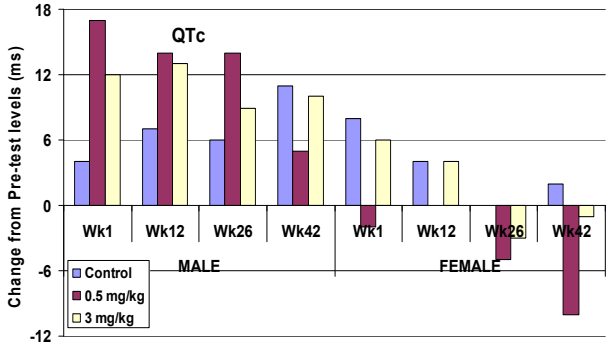
No ophthalmoscopy data were submitted. It was reported that there were no treatment related ocular changes.

ECG
Examinations of all animals were performed pre-treatment and during Weeks 1, 12, 25 and 42. Examinations were performed approximately 1.5-2 hours post-dose.

When compared with pre-treatment values, decreased heart rate was observed up to Week 26, but not in Week 42 in M and up to Week 12, but not in Weeks 26 and 42 in F. QTc was increased in M up to Week 26, but not in Week 42, whereas there was no apparent change in F values. In addition, tall t-waves were observed in 3 and 5 M and 1 and 4 F at control and HD, respectively.

Figures of FTY720 effect on heart rate and QTc





Respiration

Auscultations were performed concurrently with ophthalmoscopy examinations in Week 43, under anesthesia.

No data were submitted. The Sponsor reported that there were no treatment related effects during respiratory auscultation.

Hematology

Blood samples were taken from all animals pre-treatment and in Weeks 6, 12, 25 and 39 of treatment and Weeks 4, 11 and 24 of the recovery period. The following parameters, listed in the study report, were assessed.

erythrocytes	Wintrobe indices	white blood cell count
hematocrit	red cell distribution width (RDW)	white blood cell differential
hemoglobin	reticulocytes	platelets
PT (prothrombin time)	APTT (activated partial thromboplastin time)	

In M, WBC (45-59% and 55-65%) and lymphocyte (78-86% and 82-88%) counts decreased at the LD and HD, respectively. Similarly, in F, a decrease in WBC (25-44% and 32-54%) and lymphocyte (56-71% and 77-79%) count was observed at the LD and HD, respectively. Neutrophils decreased over the first 12 weeks in M (1-13% and 9-10%) and in F (25-31% and 27%) at the LD and HD, respectively. WBC in animals at the LD and lymphocytes in animals at the LD and HD reversed after 26 weeks recovery; however, WBC count was still decreased in animals at the HD by 24% (M) and 47% (F). Neutrophil levels recovered.

Clinical Chemistry

Blood samples were taken from all animals pre-treatment and in Weeks 6, 12, 25 and 39 of treatment and Weeks 4, 11 and 24 of the recovery period. The following parameters, listed in the study report, were assessed.

alanine aminotransferase	globulins (G)	chloride
alkaline phosphatase	glucose	calcium
aspartate aminotransferase	urea	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	sodium	cholesterol
albumin (A)	potassium	A/G ratio

Decrease from pre-treatment levels occurred from Week 6 for total protein and globulin that resulted in an increase in A/G ratio of 28% and 27% (M) and 17% and 18% (F) by Week 39 at the LD and HD, respectively. Total protein levels reversed after 4 weeks of recovery; globulin and A/G recovered by Week 24.

Urinalysis

Samples were taken from all animals pre-treatment and in Weeks 6, 12, 25 and 39 of treatment and Weeks 4, 11 and 24 of the recovery period. The following parameters, listed in the study report, were assessed.

specific gravity	glucose*	protein*
bilirubin*	ketones*	urobilinogen*
blood*	pH*	

*test strip determinations

There were no treatment-related changes in urinalysis over the course of treatment.

Gross Pathology

Gross pathology was assessed in all animals.

Macroscopic findings were observed in the digestive system including depressed, raised and red foci. After 13 weeks of recovery, red foci were still observed in the digestive system. At the HD, 1 M had enlarged testes and 1 F had a dark red uterus. Observations in the lung included red foci in 1 and 1 M at the LD and HD, respectively, and at the HD; lungs were not collapsed in 2 M and 1 F.

Organ Weights

At necropsy the following organs, listed in the study report, were weighed.

adrenals*	ovaries*	liver
brain	pituitary	thyroid with parathyroids*
heart	prostate	uterus
kidneys*	lungs	thymus
testes*		

*paired organs weighed together

In M, relative weight increases included brain (22%), lungs (58%) and thymus (160%) and the thyroid (37%) at the LD. At the HD, the brain (12%), lungs (57%) and thymus (171%) weights were increased and thyroid (31%) weight was decreased. No recovery, at Week 13, was observed for thymus weight at the HD and only partial recovery of lung weight at the HD.

In F, an increase in relative weight was observed in the heart (6%), kidney (13%) and liver (9%) at the LD. At the HD, heart (11%), kidney (10%), liver (10%) and lungs (20%) weights were increased. No recovery, at Week 13, was observed for the change in heart weight at the LD and HD or kidney weight at the HD and only partial recovery of lung weight at the HD.

Histopathology

At necropsy, the following organs, as listed in the study report were prepared for histopathology examination. Tissues were fixed in 10% formalin, except for testes and epididymis that were fixed in Bouin's solution and eye balls that were fixed in 3% Sorensen's buffered glutaraldehyde solution. Lungs were stained with Masson Trichrome to evaluate pulmonary increased collagen.

adrenals	jejunum	sciatic nerve
animal identification	kidneys	seminal vesicle
aorta	lacrimal glands	skeletal muscle
bone marrow (in bone)	liver	skin
brain	lungs	spinal cord
cecum	lymph node: bronchial	spleen
cervix	lymph node: mandibular	sternum
colon	lymph node: mesenteric	stomach
duodenum	macroscopic lesions	testes
epididymides	mammary gland	thymus
esophagus	ovaries	thyroid with parathyroids
eye	pancreas	tongue
femur(distal w/joint)	pituitary	trachea
gall bladder	prostate	urinary bladder
heart	rectum	uterus
ileum	salivary glands	vagina

Adequate Battery: Yes
Peer Review: All assessments were peer reviewed as per SOPs.

In lungs, distended alveoli were observed and only partially recovered after 13 weeks, increased collagen and smooth muscle hypertrophy that only partially recovered after 26 weeks and decreased collapse of the lung in M at the LD. Stomach had findings of minimal to slight mononuclear infiltration and hyperplasia was observed in the other GI tract organs, duodenum and ileum. Lymphoid organ findings were atrophy and lymphocyte depletion. F displayed few histological changes, but did have findings in the lung that included alveolus distended, focal, Increase collagen, decreased collapse, smooth muscle hypertrophy and congestion.

Summary of major male microscopic findings

		Main			13 Wk Rec.			26 Wk Rec.		
Tissue	**Finding**	**0**	**0.5**	**3**	**0**	**0.5**	**3**	**0**	**0.5**	**3**
Brain	Lymphocyte infiltration									
	minimal:	**1/4**	**1/4**	**0/4**	**1/3**	**2/3**	**0/3**	**0/3**	**2/3**	**1/3**
	slight:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**1/3**
	Gliosis									
	minimal:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	slight:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**1/3**
	Chronic inflammation									
	minimal:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	slight:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**1/3**
Pituitary gland	Hemorrhage, pars nervosa									
	minimal:	**0/3**	**1/4**	**0/4**	**0/3**	**0/3**	**0/2**	**0/3**	**0/3**	**0/2**
	slight:	**0/3**	**0/4**	**1/4**	**0/3**	**0/3**	**0/2**	**0/3**	**0/3**	**0/2**
Heart	Mononuclear infiltrate									
	minimal:	**2/4**	**4/4**	**4/4**	**1/3**	**2/3**	**3/3**	**3/3**	**1/3**	**1/3**
	slight:	**0/4**	**0/4**	**0/4**	**0/3**	**1/3**	**0/3**	**0/3**	**1/3**	**0/3**
Lungs	Mononuclear infiltrate									
	minimal:	**3/4**	**1/4**	**1/4**	**0/3**	**2/3**	**2/3**	**0/3**	**2/3**	**0/3**
	slight:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**1/3**	**0/3**	**0/3**	**0/3**
	Pleura thickened									
	minimal:	**1/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	slight:	**1/4**	**0/4**	**1/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	Alveolus distended									
	minimal:	**0/4**	**2/4**	**1/4**	**0/3**	**1/3**	**1/3**	**0/3**	**0/3**	**0/3**
	slight:	**1/4**	**0/4**	**3/4**	**0/3**	**0/3**	**1/3**	**0/3**	**0/3**	**0/3**
	moderate:	**0/4**	**1/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	Increase collagen									
	minimal:	**1/4**	**4/4**	**1/4**	**0/3**	**0/3**	**2/3**	**0/3**	**1/3**	**0/3**
	slight:	**0/4**	**0/4**	**2/4**	**0/3**	**0/3**	**0/3**	**0/3**	**1/3**	**2/3**
	Decreased collapse									
	present:	**0/4**	**0/4**	**0/4**	**1/3**	**0/3**	**1/3**	**0/3**	**0/3**	**0/3**
	minimal:	**0/4**	**2/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	Smooth muscle hypertrophy									
	minimal:	**0/4**	**2/4**	**2/4**	**0/3**	**0/3**	**1/3**	**1/3**	**0/3**	**0/3**
	slight:	**0/4**	**0/4**	**2/4**	**0/3**	**0/3**	**1/3**	**0/3**	**0/3**	**2/3**
	Thrombus									
	minimal:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	slight:	**0/4**	**0/4**	**1/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	Subacute inflammation									
	minimal:	**0/4**	**0/4**	**0/4**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
	slight:	**0/4**	**0/4**	**1/4**	**0/3**	**0/3**	**1/3**	**0/3**	**0/3**	**0/3**

Tissue	Finding	Main			13 Wk Rec.			26 Wk Rec.		
		0	0.5	3	0	0.5	3	0	0.5	3
Liver	Necrosis									
	minimal:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	1/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	1/3
	Mononuclear infiltration									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	1/3	0/3	1/3	1/3
	Subacute inflammation									
	minimal:	0/4	0/4	0/4	0/3	0/3	1/3	0/3	0/3	0/3
	Periportal inflammation									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	1/3
Stomach	Mononuclear infiltrate, pylorus									
	minimal:	1/4	0/4	0/4	0/3	0/3	0/3	1/3	0/3	0/3
	slight:	0/4	1/4	1/4	0/3	1/3	0/3	0/3	3/3	0/3
	Mononuclear infiltrate, fundus									
	minimal:	0/4	1/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	2/4	0/3	0/3	1/3	0/3	0/3	1/3
	Mononuclear infiltrate, cardia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	1/4	0/3	0/3	0/3	0/3	1/3	1/3
	Gland dilatation									
	minimal:	0/4	2/4	0/4	1/3	0/3	2/3	1/3	0/3	1/3
	slight:	0/4	1/4	0/4	0/3	1/3	0/3	1/3	1/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	1/3	1/3	0/3	0/3
	Erosion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	1/3
	Hemorrhage									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	1/3
Duodenum	Lymphoid hyperplasia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Ileum	Lymphoid hyperplasia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Cecum	Crypt dilatation									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Adrenal gland	Extracapsular cortical									
	minimal:	1/4	0/4	0/4	0/3	0/3	1/3	0/3	1/3	1/3
	slight:	1/4	1/4	0/4	0/3	2/3	1/3	0/3	1/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Mononuclear infiltrate									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3

Tissue	Finding	Main			13 Wk Rec.			26 Wk Rec.		
		0	0.5	3	0	0.5	3	0	0.5	3
Spleen	W pulp lymphocyte depletion									
	minimal:	0/4	4/4	2/4	0/3	1/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	1/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Polymorphonuclear infiltrate									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Thickened capsule									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphoid hyperplasia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	1/3	0/3	1/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	1/3	0/3	0/3	0/3
Thymus	Involution									
	minimal:	0/4	0/4	1/4	0/3	1/3	2/3	1/3	2/3	2/2
	slight:	1/4	1/4	1/4	1/3	2/3	0/3	1/3	0/3	0/2
	moderate:	2/4	0/4	0/4	1/3	0/3	0/3	0/3	0/3	0/2
	marked:	0/4	0/4	0/4	0/3	0/3	1/3	0/3	0/3	0/2
	Cyst									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/2
	slight:	0/4	1/4	1/4	0/3	0/3	1/3	0/3	0/3	0/2
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	1/3	0/3	0/3	0/3	0/2
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/2
Bronchial Lymph Node	Macrophage pigment									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	0/4	1/4	2/3	1/3	2/3	0/3	0/3	1/3
	Lymphocyte depletion									
	minimal:	0/4	2/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Follicular hyperplasia									
	minimal:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphangiectasis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	2/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
Mandibular Lymph Node	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	1/3	0/3	0/3
	slight:	0/4	1/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Polymorphonuclear infiltrate									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphocyte depletion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3

Tissue	Finding	Main 0	Main 0.5	Main 3	13 Wk Rec. 0	13 Wk Rec. 0.5	13 Wk Rec. 3	26 Wk Rec. 0	26 Wk Rec. 0.5	26 Wk Rec. 3
	Necrosis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
Mandibular	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Lymph Node	Lymphangiectasis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
Mesenteric	Sinus histiocytosis									
Lymph Node	minimal:	1/4	0/4	0/3	0/3	1/3	0/3	1/3	0/3	0/3
	slight:	0/4	2/4	0/3	2/3	0/3	1/3	1/3	0/3	0/3
	Macrophage pigment									
	minimal:	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	2/4	1/3	2/3	0/3	1/3	0/3	0/3	0/3
	Follicular hyperplasia									
	minimal:	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	2/4	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphocyte depletion									
	minimal:	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	Necrosis									
	minimal:	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphangiectasis									
	minimal:	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	1/3
	slight:	1/4	0/4	0/3	0/3	0/3	0/3	1/3	2/3	1/3
Kidneys	Mononuclear infiltrate									
	minimal:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	2/3	1/3	0/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	1/3	0/3	0/3	0/3	0/3
	Tubular cast, papilla									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	1/3
Epididymides	Oligospermia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	2/4	0/4	0/3	1/3	0/3	0/3	1/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	1/3

Rec. - recovery

Summary of major female microscopic findings with FTY720

Tissue	Finding	Main 0	Main 0.5	Main 3	13 Wk Rec. 0	13 Wk Rec. 0.5	13 Wk Rec. 3	26 Wk Rec. 0	26 Wk Rec. 0.5	26 Wk Rec. 3
Brain	Lymphocyte infiltration									
	minimal:	0/4	2/4	1/4	0/3	1/3	0/3	1/3	3/3	2/3
Sciatic nerve	Mononuclear infiltration									
	minimal:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Spinal cord	Lymphocyte infiltration									
	minimal:	0/4	1/4	0/4	0/3	0/3	0/3	1/3	0/3	1/3

Tissue	Finding	Main 0	Main 0.5	Main 3	13 Wk Rec. 0	13 Wk Rec. 0.5	13 Wk Rec. 3	26 Wk Rec. 0	26 Wk Rec. 0.5	26 Wk Rec. 3
Heart	Mononuclear infiltrate									
	minimal:	4/4	4/4	4/4	3/3	0/3	0/3	1/3	2/3	1/3
	slight:	0/4	0/4	0/4	0/3	2/3	1/3	0/3	0/3	0/3
	Epicardium inflammation									
	minimal:	0/4	0/4	0/4	0/3	1/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	Myocardium vacuolation									
	minimal:	0/4	0/4	0/4	0/3	1/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	1/3	0/3	0/3	0/3	0/3
Lungs	Mononuclear infiltrate									
	minimal:	0/4	1/4	1/4	0/3	1/3	0/3	0/3	1/3	1/3
	slight:	0/4	0/4	0/4	0/3	1/3	0/3	0/3	0/3	0/3
	Alveolus distended									
	minimal:	1/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	0/4	2/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
	Increase collagen									
	minimal:	2/4	1/4	0/4	1/3	0/3	1/3	0/3	0/3	0/3
	slight:	0/4	0/4	2/4	0/3	1/3	1/3	0/3	0/3	1/3
	Decreased collapse									
	present:	0/4	0/4	0/4	0/3	0/3	1/3	0/3	0/3	0/3
	minimal:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Smooth muscle hypertrophy									
	minimal:	0/4	0/4	1/4	1/3	1/3	1/3	0/3	1/3	1/3
	slight:	0/4	0/4	2/4	0/3	0/3	0/3	0/3	0/3	0/3
	Vascul. S.muscle hypertrophy									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
	Subacute inflammation									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Chronic inflammation									
	minimal:	1/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Adrenal gland	Extracapsular cortical									
	minimal:	0/4	0/4	1/4	0/3	1/3	0/3	0/3	1/3	2/3
	slight:	0/4	0/4	0/4	1/3	0/3	1/3	1/3	1/3	0/3
Spleen	W. pulp lymphocyte depletion									
	minimal:	0/4	0/4	3/4	1/3	1/3	0/3	0/3	0/3	0/3
	slight:	1/4	2/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
Thymus	Involution									
	minimal:	1/4	2/4	4/4	2/3	0/3	2/3	2/3	2/3	1/3
	slight:	1/4	1/4	0/4	1/3	3/3	1/3	0/3	1/3	0/3
	Cyst									
	minimal:	2/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	2/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3
	moderate:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3

Tissue	Finding	Main 0	Main 0.5	Main 3	13 Wk Rec. 0	13 Wk Rec. 0.5	13 Wk Rec. 3	26 Wk Rec. 0	26 Wk Rec. 0.5	26 Wk Rec. 3
Bronchial Lymph Node	Macrophage pigment	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	minimal:	1/4	0/4	0/3	2/3	2/3	1/3	2/3	2/3	2/3
	slight:	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	1/3
	moderate:									
	Follicular hyperplasia	1/4	1/4	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	minimal:									
	Lymphangiectasis	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	1/3
	minimal:	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3	0/3
	slight:									
Bronchial Lymph Node	Lymphocyte depletion	0/4	3/4	2/3	0/3	0/3	0/3	0/3	0/3	0/3
	minimal:	0/4	1/4	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	slight:									
Mandibular Lymph Node	Lymphangiectasis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	1/3	0/3	0/3
	Sinus histiocytosis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	0/4	1/3	0/3	0/3	0/3	0/3	0/3
	Congestion									
	minimal:	0/4	0/4	0/4	1/3	0/3	0/3	0/3	0/3	0/3
	Polymorphonuclear infiltrate									
	minimal:	0/4	1/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	1/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
Mesenteric Lymph Node	Macrophage pigment									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	2/4	1/2	2/4	0/3	2/3	1/3	0/3	0/3	0/3
	Follicular hyperplasia									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	1/4	2/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	Lymphangiectasis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	1/3	0/3	0/3
	slight:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	2/3	3/3
	Sinus histiocytosis									
	minimal:	0/4	0/4	0/4	0/3	0/3	0/3	0/3	1/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	1/3	0/3
	Lymphocyte depletion									
	minimal:	0/4	1/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3
	slight:	0/4	0/4	1/4	0/3	0/3	0/3	0/3	0/3	0/3

Rec. – recovery, Vascul. – vascular.

Serology

Blood samples were taken from all animals during Week 39 and during recovery Week 24.

Although changes occurred in results at the end of treatment compared pre-treatment results (from the Vendor), there were no consistent treatment-related changes in measles or CMV compared to control animals. In contrast, there was an increase in positive results for hepatitis IgG in treated animals with 4 and 1 LD and HD, respectively, becoming positive during treatment.

Immune function
Blood and spleen samples were taken in Weeks 42-43 (6 and 13 Jan 2000) for *in vitro* analysis of the immune function.

The proliferative responses of B- and T-cells were suppressed at both doses by 52.1-96% and 88.9-97.2% compared to control animals, respectively. In contrast, the proliferative responses of B-cells in the spleen were not different from control responses and T-cell responses were moderately suppressed by 24.9-41.2%. The mixed lymphocyte response in the treated animals was suppressed compared to control animals. There was no obvious suppression of natural killer cell activity with treatment.

Immunoglobulins
Blood samples were taken in Weeks 42 (6 Jan 2000) for immunoglobulin analysis.

There were no treatment related changes in IgM or IgA levels observed. No conclusion can be made concerning IgG and IgE levels as they were below the level of detection.

Toxicokinetics
Blood samples were taken on Day 1-2 and in Weeks 13 and 40 at 1, 2, 4, 8, 12 and 24 hours from main study animals. Blood samples were taken from recovery animals at scheduled termination. Brain samples were taken from the frontal lobe of the cerebral cortex from all scheduled necropsies. Levels of FTY720, M2 and M3 were measured.

Generally, there were no sex differences. Plasma exposure to FTY720 was greater than dose-proportional with accumulation over the course of the study. T_{max} values were 2-8 hours. Metabolites M2 and M3 were also assessed and generally there were no sex differences.

Brain-to-blood ratios were high, i.e., 316 and 1,294 at the LD and HD, respectively. Elimination of FTY720 from brain was slow; brain levels were 34 and 375 ng/ml at the LD and HD, respectively, in Week 26 of recovery.

Table of FTY720 toxicokinetic parameters (Sponsor's)

dose	0.5 mg/kg/day			3.0 mg/kg/day		
time	Day 1-2	Week 13	Week 40	Day 1-2	Week 13	Week 40
			males			
t_{max1}	8	4	4	4	8	2
C_{max2}	9.96	19.96	21.69	49.80	126.46	125.37
C_{max}/dose[3]	19.9	39.9	43.4	16.6	42.2	41.8
$AUC_{(0\text{-}24h)}$[4]	187	403	420	886	2473	2536
$AUC_{(0\text{-}24\ h)}$/dose[5]	375	806	840	295	824	845
			females			
t_{max1}	4	4	8	4	4	4
C_{max2}	11.51	19.83	18.77	52.66	191.43	122.89
C_{max}/dose[3]	23.0	39.7	37.5	17.6	63.8	41
$AUC_{(0\text{-}24h)}$[4]	220	396	385	952	3491	2543
$AUC_{(0\text{-}24\ h)}$/dose[5]	439	792	771	317	1164	848

[1]hour, [2]ng/mL, [3](ng/mL)/(mg/kg/day), [4](h·ng/mL), [5](h·ng/mL)/(mg/kg/day)

Table of M2 and M3 toxicokinetic parameters (Sponsor's)

metabolite	M2			M3		
time	Day 1-2	Week 13	Week 40	Day 1-2	Week 13	Week 40
			males			
t_{max1}	4	2	2	4	4	8
C_{max2}	21.21	31.36	21.93	38.78	79.61	46.53
C_{max}/dose[3]	7.07	10.45	7.31	12.93	26.54	15.51
$AUC_{(0-24h)}$[4]	256	472	328	567	1316	899
$AUC_{(0-24\ h)}$/dose[5]	85.4	157.4	109.4	189	439	300
			females			
t_{max1}	2	2	2	4	4	4
C_{max2}	28.31	65.24	26.33	28.35	114.64	66.51
C_{max}/dose[3]	9.44	21.75	8.78	9.45	38.21	22.17
$AUC_{(0-24h)}$[4]	272	741	366	395	1596	1099
$AUC_{(0-24\ h)}$/dose[5]	90.6	247.1	122.1	132	532	366

[1]hour, [2]ng/mL, [3](ng/mL)/(mg/kg/day), [4](h·ng/mL), [5](h·ng/mL)/(mg/kg/day)

Table of blood and brain FTY720 concentration (Sponsor's)

Dose	Gender	Period	Mean conc. of FTY720 [ng/mL]*				FTY720
[mg/kg/day]			In blood	stdev	In brain	stdev	C_{tissue}/C_{blood}
0.5	Male	End of treatment	13.20	4.81	4003	1680	303
0.5	Female	End of treatment	12.18	2.48	4010	887	329
0.5	Male	13 Week recovery	0.00	0.00	360	89	**
0.5	Female	13 Week recovery	0.01	0.02	222	189	22200
0.5	Male	26 Week recovery	0.02	0.03	28	25	1400
0.5	Female	26 Week recovery	0	0	40	36	**
3.0	Male	End of treatment	75.77	10.67	112077	30699	1479
3.0	Female	End of treatment	81.40	29.97	90212	21284	1108
3.0	Male	13 Week recovery	0.18	0.09	8393	2483	46628
3.0	Female	13 Week recovery	0.28	0.08	4326	895	15450
3.0	Male	26 Week recovery	0.06	0.05	413	405	6883
3.0	Female	26 Week recovery	0.16	0.07	337	75	2106

*Concentration in the brain set to ng/mL with 1 g = 1 mL, **Not applicable;

Stability and Homogeneity

FTY720 was chemically stable for at least 40 days at 6°C and at least 4 hours at room temperature. Samples were taken from target solutions of 0.05 and 2 mg/ml at different time points that included; initial, 4 hours stirring at room temperature, 21 and 40 Days at 6°C. Stability data showed that the 0.05 and 2 mg/ml solutions ranged between 93-102% of the target concentration after 4 hours at room temperature and ranged between 95-102% of the target concentration after 40 days at 6°C. The Sponsor stated that "*Formulation uniformity testing was not required because FTY720 was formulated as solution*".

Study title: Y32720: Toxicity to Cynomolgus monkeys by repeated oral administration for 52 weeks

Study no.:	PCS-r971546
Study report location:	EDR: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12 March 1997
GLP compliance:	Yes ((b) (4) , 1997)
QA statement:	Yes (Dated: 16 March 1999)
Drug, lot #, and % purity:	Y32720, Batches № S, 100%

Key Study Findings

- Labored breathing was observed in one 10 mg/kg/day male and in animals at greater than 3 mg/kg/day while under sedation.
- Decreased body weights occurred in all groups and food consumption in males at greater than 3 mg/kg/day and females at 10 mg/kg/day.
- Decreased white blood cells and lymphocytes occurred in all treated groups.
- The main macroscopic findings observed in all treated groups included lungs not collapsed and enlarged hearts.
- Smooth muscle cell hyperplasia and increased collagen in the walls of bronchioles and alveolar ducts and the entrance to alveolar sacs, with macrophage aggregates in the alveoli of half of the animals was observed in the lungs in 10 mg/kg/day animals. Smooth muscle hypertrophy was observed in all treatment groups, macrophage infiltration or aggregation was observed in lungs at greater than 3 mg/kg/day.
- Other histopathological findings observed at all treatment levels included myocarditis, myocardial vacuolation, degeneration and hemorrhage, ventricular endocardial fibrosis, hypertrophy, slight valvular endocarditis and arterial wall thickening of the heart. Pituitary gland findings occurred only in males and included vacuolation and fibrosis. Nerve fiber degeneration was observed in the spinal cord and sciatic nerve
- Males and females had similar plasma exposure at 24 hours post-dose.
- A NOAEL was not established.

Methods

Doses:	0, 1, 3 and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg
Formulation/Vehicle:	Distilled de-ionized water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group	
Main study:	4
Age:	13 months to 2 years at start of treatment
Weight:	1.7-2.3 kg

Observations and Results

Mortality

There were no unscheduled deaths in any group.

Clinical Signs

Observations were performed regularly throughout the working day (09:00-17:00 hours).

Vomiting and salivation were observed in all treatment groups and were more prevalent in M. Subdued/huddled behavior occurred in 2 and 1 M at the MD and HD, respectively, and in 2 F at the MD. Breathing signs were observed at the MD and HD and included rapid breathing in 1 M at the HD, labored breathing in 1 M at the HD and labored breathing while under ketamine sedation in 2 M at the HD, 1 F at the MD and all F at the HD.

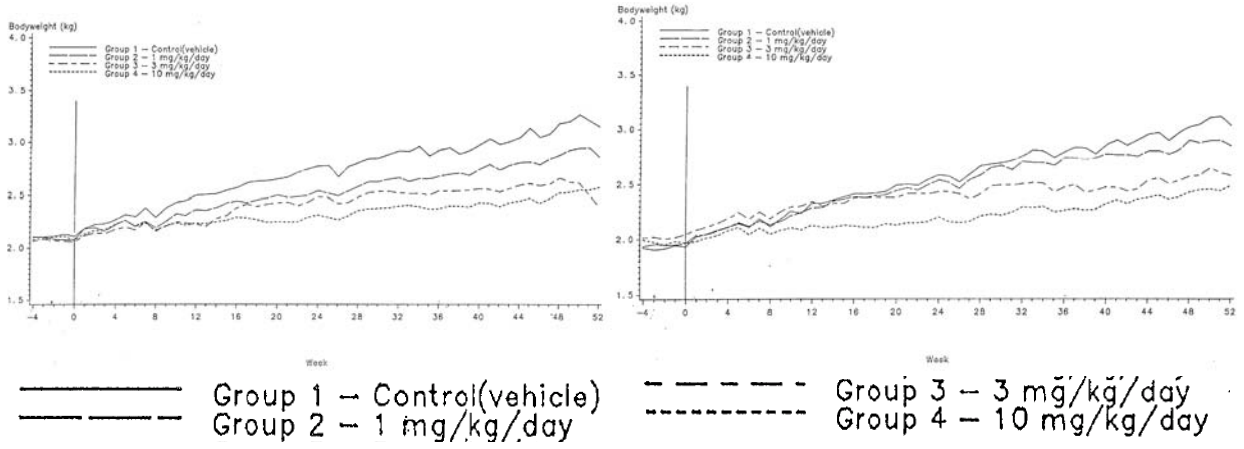
Body Weights

Body weights were recorded weekly prior to treatment and during treatment.

There were dose-dependent decreases in body weights of M and F. The final mean total change in body weight compared to control animals were decreases of 25%, 67% and 52% for in M at the LD, MD and HD, respectively, and 20%, 52% and 53% in F at the LD, MD and HD, respectively.

Figures of body weights (Sponsor's)

MALE **FEMALE**



Food Consumption

Food consumption was calculated daily, during the experimental period, by recording the quantity of food left by individual animals.

Over the first month, a decrease in mean total food consumption was observed in M at the LD (11%), MD (36%) and HD (34%), respectively, compared with control M. By the third month, M at the LD had recovered and had a similar food consumption to control M; however, food consumption in M at the MD and HD was still decreased by 22% and 26%, respectively, and remained decreased. By Week 52, total food consumption was decreased by 9 % and 16% in M at the MD and HD, respectively, compared to control

animals. Compared to control F, there was no change of total food consumption at the LD and MD; however, there was a decrease in total food consumption at the HD that started at about Week 10 and resulted in a 23% decrease.

Ophthalmoscopy and hearing
Examinations of all animals were performed pre-treatment and during Weeks 26 and 52/53.

Two F at the MD had anterior capsular opacity of the right eye, 1 F at the HD had anterior capsular opacity in both eyes and 1 F at the HD had corneal opacity in the left eye at Week 52.

ECG
Examinations of all animals were performed pre-treatment and during Weeks 26 and 52. Examinations were performed pre-dose.

The Sponsor states that "*there were no abnormalities of the electrocardiographic wave-form at Weeks 26 or 52 which could be attributed to treatment*", however no data were submitted. Recordings were performed pre-dose; therefore the data only represent the chronic effect of FTY720 on heart rate.

Hematology
Blood samples were taken from all animals pre-treatment and in Weeks 13, 26, 39 and 52 of treatment. The following parameters were assessed: PCV,HGB, RBC count, MCHC, MCV, total WBC count, neutrophils, lymphocytes, eosinophils, basophils, monocytes, LUC, platelets, reticulocyte count, PT and APTT.

Reticulocytes increased in all treated M and F, the largest increases, 165-300%, were observed early in treatment (Week 13) in M at the MD, at Week 26 in all treated F and at the end of treatment in M at the HD. WBC counts decreased in all treated animals (24%-86%) and mainly resulted from a decrease in lymphocytes; however, basophils, monocytes and LUC were also reduced. There was an increase in PT in F at each sampling time point.

Clinical Chemistry
Blood samples were taken from all animals pre-treatment and in Weeks 13, 26, 39 and 52 of treatment. The following parameters were assessed: total protein, urea, creatine, sodium, potassium, calcium, inorganic phosphorus, chloride, total bilirubin, cholesterol, ALP, GPT, GOT, glucose, magnesium, creatine phosphokinase, LDH, triglycerides, uric acid, leucine amino peptidase, phospholipids, albumin, α_1-albumin, α_2-albumin, β-globulin, γ-globulin, total globulin and A/G ratio.

In Week 26, GPT, GOT, LDH, calcium, potassium, total protein, β-globulin were decreased in all treated M, with GOT and LDH levels still decreased in Week 52. In F, ALP, total protein, β-globulin were decreased in Week 13 and potassium was increased from Week 13; levels were still changed at Week 52

Urinalysis

Urine samples were taken from all animals pre-treatment and in Weeks 13, 26, 39 and 52 of treatment. The following parameters were assessed: pH, specific gravity, protein, sodium, potassium, chloride, magnesium, total reducing substance, glucose, ketones, bile pigments, urobilinogen, heme pigments, epithelial cells, polymorphonuclear leucocytes, mononuclear leucocytes, erythrocytes, organisms, renal tubule casts, other abnormal constituents including: sperm and protozoa.

There were no treatment-related changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

The main macroscopic findings included lungs not collapsed in 2 and 4 M at the MD and HD and in 1, 1, 3 and 3 F at the control, LD, MD and HD, respectively. Enlarged hearts were observed in 1 M at the LD, MD and HD and 1 F at the MD. In addition, 1 M at the MD showed evidence of widespread infection in the GI tract.

Organ Weights

At necropsy the following organs, listed in the study report were weighed.

adrenals	ovaries	seminal vesicles
brain	pancreas	spleen
heart	pituitary	testes (with epididymides)
kidneys	prostate	thymus
liver	salivary glands	thyroids (with parathyroids)
lungs	(submandibular)	uterus

In M, increases in relative weights included the lungs (31%, 47% and 58%), heart (31%, 37% and 32%), pituitary gland (39%, 33% and 33%) and adrenals (44%, 30% and 27%) and a decrease in spleen (21%, 26% and 40%), pancreas (10%, 17% and 28%) and thymus (9%, 15% and 35%) weight at the LD, MD and HD, respectively.

In F, an increase in relative weights was observed in lungs (11%, 59% and 77%) and heart (11%, 42% and 11%) and a decrease in spleen (16%, 36% and 37%) and thymus (25%, 26% and 37%) at the LD, MD and HD, respectively. There was also a 75% increase in gonad weights at the HD in F.

Electron Microscopy

Sections of liver, lung, kidney (cortex, medulla and papilla) and brain were taken from all animals and prepared for electron microscopy.

After electron microscopy examination of the lungs, smooth muscle cell hyperplasia and increased collagen were observed in the walls of bronchioles and alveolar ducts and the entrance to alveolar sacs of all 4 chosen animals at the HD (2 M and 2 F). Further, in 50% of examined animals at the HD (1 M and 1 F), the alveoli had macrophage aggregates.

Histopathology

At necropsy, the following organs, as listed in the study report, were prepared for histopathology examination. Tissues were fixed in 10% formalin, except for the eyes that were fixed in Davidson's fixative. In addition, pieces of liver and kidney were placed in formol calcium. Selected lungs/bronchi were stained with Trichrome and van Gieson stains at the discretion of the pathologist.

adrenals	liver	spinal cord (cervical, thoracic and lumbar regions)
alimentary tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	lungs (with bronchi)	spleen
	lymph nodes (cervical and mesenteric)	sternum
	mammary gland	testes (with epididymides)
aorta (arch and abdominal)	ovaries	thymus
brain (cerebrum, thalamic nuclei, midbrain, medulla and cerebellum)	pancreas	thyroids (and parathyroids)
	pituitary	tongue
	prostate	trachea
eyes (with optic nerve)	salivary gland (submandibular)	urinary bladder
femur (with joint)	sciatic nerve	uterus
gall bladder	seminal vesicles	vagina
heart	skeletal muscle (quadriceps femoris)	
kidneys	skin	
lachrymal gland		

Adequate Battery: Yes

Peer Review: Study pathologist was [redacted] (b) (4) and the results were peer reviewed by [redacted] (b) (4). There was consensus of opinions on the diagnosis.

Histopathological observations in all groups included lymphoid organ atrophy consistent with the pharmacological action of FTY720. Other observations included heart findings at all treatment levels, very slight to slight myocarditis, myocardial vacuolation, degeneration and hemorrhage; there was also very slight to moderate ventricular endocardial fibrosis, hypertrophy, slight valvular endocarditis and arterial wall thickening was present in most treated animals. In addition, aorta findings of slight subendothelial fibrosis and moderate intimal thickening were observed in M at the HD. The very slight to marked smooth muscle hypertrophy, in all treatment groups, was dose-dependent; increase in the severity of macrophage infiltration or aggregation was observed in the lungs of animals at the MD and HD, as was alveolar hyperdistension and bronchiolar epithelial extension. Pituitary gland findings occurred only in M and included vacuolation, fibrosis at the MD and hypertrophy at the HD. Nerve fiber degeneration was observed in the spinal cord and sciatic nerve and moderate optic nerve arteritis in 1 F at the MD. Slight zone fasciculate hypertrophy and mineralization was observed in M adrenal glands and bone marrow myeloid hyperplasia was observed in all treated animals.

Summary of major microscopic findings

Tissue	Findings	MALE 0	MALE 1	MALE 3	MALE 10	FEMALE 0	FEMALE 1	FEMALE 3	FEMALE 10
Pituitary gland	Peripheral fibrosis								
	very slight	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Pars nervosa vacuolation								
	present:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Pars distalis hypertrophy								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Nerves	Spinal cord fiber degeneration								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Sciatic nerve fiber degeneration								
	very slight:	2/4	0/4	0/4	2/4	0/4	1/4	1/4	0/4
	slight:	0/4	0/4	2/4	1/4	0/4	0/4	0/4	0/4
	Optic nerve arteritis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
Eyes	Subrentinal inflammatory cells								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Swollen lenticular fibers								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4
Muscle	Myofiber necrosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Heart	Myocarditis								
	very slight:	2/4	1/4	3/4	0/4	1/4	0/4	2/4	0/4
	slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
	Myocardial vacuolation								
	very slight:	1/4	2/4	0/4	1/4	0/4	0/4	1/4	1/4
	slight:	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
	Myocardial degeneration								
	very slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Myocardial hemorrhage								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Myocardial fibrosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4
	Myocardial necrosis								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Ventricular endocarditis								
	very slight:	1/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Ventricular endocardial fibrosis								
	very slight:	0/4	3/4	2/4	1/4	0/4	1/4	1/4	0/4
	slight:	0/4	1/4	0/4	1/4	0/4	2/4	1/4	0/4
	moderate:	0/4	0/4	1/4	2/4	0/4	1/4	1/4	0/4
	marked:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Tissue	Findings	MALE 0	MALE 1	MALE 3	MALE 10	FEMALE 0	FEMALE 1	FEMALE 3	FEMALE 10
Heart	Ventricular endocardial hypertrophy								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	3/4	2/4	2/4	0/4	3/4	2/4	3/4
	moderate:	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
	marked:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	Ventricular Interstitial swelling								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Atrial endocarditis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	Atrial myocarditis								
	very slight:	0/4	0/4	0/4	0/4	0/4	1/4	1/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Atrial myocardial hypertrophy								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Endocardial hemorrhage								
	very slight:	0/4	1/4	0/4	0/4	0/4	2/4	0/4	0/4
	Valvular endocarditis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Arterial mural thickening								
	present:	0/4	3/4	3/4	4/4	1/4	1/4	2/4	3/4
Aorta	Subendothelial fibrosis								
	very slight	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Intimal thickening								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Lungs	Smooth muscle hypertrophy								
	very slight:	0/4	1/4	0/4	0/4	0/4	2/4	0/4	0/4
	slight:	0/4	3/4	3/4	1/4	0/4	1/4	2/4	0/4
	moderate:	0/4	0/4	1/4	3/4	0/4	0/4	2/4	3/4
	marked:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Macrophage infiltration								
	very slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
	Macrophage aggregation								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	2/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Pneumonitis								
	very slight:	4/4	3/4	3/4	2/4	3/4	3/4	3/4	1/4
	slight:	0/4	1/4	1/4	2/4	1/4	1/4	0/4	2/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	1/4
	Lymphoid tissue								
	very slight:	3/4	1/4	1/4	3/4	1/4	1/4	1/4	2/4
	slight:	1/4	0/4	1/4	0/4	2/4	1/4	0/4	1/4
	Pleural lymphoid aggregates								
	very slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4

Tissue	Findings	MALE 0	MALE 1	MALE 3	MALE 10	FEMALE 0	FEMALE 1	FEMALE 3	FEMALE 10
Lungs	Alveolar hyperdistension								
	very slight:	0/4	0/4	3/4	2/4	0/4	1/4	3/4	0/4
	slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	4/4
	Extension bronchiolar epithelium								
	very slight:	0/4	0/4	1/4	1/4	0/4	0/4	0/4	1/4
Liver	Parenchymal inflammatory cell								
	very slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
	Hepatocyte vacuolation								
	very slight:	0/4	0/4	0/4	0/4	1/4	1/4	0/4	0/4
	slight:	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	2/4	0/4	0/4
	Hepatocyte hypertrophy								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
	Hemorrhage/Inflammation								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
	Hepatocyte fat								
	very slight:	0/4	1/4	3/4	1/4	1/4	3/4	1/4	1/4
	slight:	0/4	0/4	0/4	1/4	2/4	0/4	0/4	0/4
	moderate:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Subcap. Fibrosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Subcap. hemorrhage/necrosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	Subcap. hemorrhage/Inflammation								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Stomach	Mucosal hemorrhage								
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	moderate:	0/4	1/4	0/4	0/4	1/4	0/4	0/4	0/4
	Inflammatory cells infiltration								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Kidneys	Cortical inflammatory cells								
	very slight:	1/4	0/4	1/4	3/4	0/4	1/4	0/4	0/4
	slight:	1/4	1/4	0/4	0/4	1/4	0/4	2/4	0/4
	Medullary inflammatory cells								
	very slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Cortical tubular basophilia								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	slight:	0/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Cortical tubular dilatation								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Tissue	Findings	MALE 0	MALE 1	MALE 3	MALE 10	FEMALE 0	FEMALE 1	FEMALE 3	FEMALE 10
Kidneys	Glomerular cell hypertrophy								
	very slight:	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4
	slight:	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
	Glomerular sclerosis								
	very slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Fat in cortical tubules								
	very slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Arteritis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	moderate:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Periartritis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
Thyroid	Ectopic thymic tissue								
	present:	0/4	1/4	0/4	1/4	1/4	0/4	1/4	0/4
	Adipose replacement								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4
	moderate:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Inflammatory cell infiltration								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Arteritis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
Adrenal gland	Zona fasciculate hypertrophy								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Mineralization								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Spleen	White pulp reduced cellularity								
	very slight:	0/4	0/4	0/4	0/4	0/4	2/4	1/4	0/4
	slight:	0/4	1/4	1/4	0/4	0/4	0/4	2/4	1/4
	moderate:	0/4	2/4	2/4	4/4	0/4	2/4	1/4	3/4
	marked:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
Thymus	Involution/atrophy								
	very slight:	0/4	4/4	1/4	0/4	0/4	2/4	2/4	0/4
	slight:	0/4	0/4	1/4	2/4	0/4	1/4	2/4	0/4
	moderate:	0/4	0/4	1/4	2/4	0/4	0/4	0/4	4/4
	marked:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	severe:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
Mesenteric Lymph Node	Lymphadenitis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	slight:	2/4	2/4	0/4	1/4	1/4	0/4	2/4	2/4
	moderate:	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4
	Sinus histiocytosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	1/4	1/4	1/4	0/4	1/4	0/4	0/4
	moderate:	1/4	3/4	1/4	1/4	1/4	3/4	4/4	4/4
	marked:	0/4	0/4	2/4	2/4	0/4	0/4	0/4	0/4

Tissue	Findings	MALE 0	MALE 1	MALE 3	MALE 10	FEMALE 0	FEMALE 1	FEMALE 3	FEMALE 10
Mesenteric Lymph Node	Lymphatic ectasia								
	very slight:	1/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	Reduced lymphoid cellularity								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	4/4	3/4	1/4	0/4	2/4	2/4	0/4
	moderate:	0/4	0/4	1/4	3/4	0/4	2/4	2/4	4/4
Cervical Lymph Node	Medullary plasmacytosis								
	very slight:	1/4	0/4	1/4	0/4	1/4	0/4	1/4	0/4
	slight:	0/4	4/4	1/4	2/4	0/4	3/4	2/4	3/4
	moderate:	0/4	0/4	0/4	2/4	0/4	1/4	1/4	1/4
	Lymphadenitis								
	very slight:	1/4	2/4	0/4	0/4	2/4	0/4	1/4	2/4
	slight:	0/4	1/4	0/4	2/4	0/4	3/4	2/4	2/4
	moderate:	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
	Sinus histiocytosis								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	slight:	0/4	4/4	2/4	2/4	0/4	3/4	2/4	3/4
	moderate:	0/4	0/4	2/4	0/4	0/4	0/4	2/4	0/4
	Reduced lymphoid cellularity								
	very slight:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	3/4	1/4	0/4	0/4	3/4	1/4	0/4
	moderate:	0/4	1/4	2/4	4/4	0/4	1/4	3/4	4/4
Bone marrow - Sternum	Myeloid hyperplasia								
	very slight:	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	slight:	0/4	3/4	2/4	1/4	2/4	2/4	0/4	0/4
	moderate:	0/4	0/4	1/4	3/4	0/4	2/4	4/4	2/4
	marked:	0/4	0/4	1/4	0/4	0/4	0/4	0/4	2/4
	Lymphoid foci								
	very slight:	0/4	0/4	1/4	0/4	1/4	0/4	0/4	0/4
	slight:	0/4	2/4	0/4	0/4	0/4	2/4	1/4	0/4
Bone marrow - Femur	Myeloid atrophy								
	present:	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Articular cartilage degeneration								
	very slight:	0/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4

Subcap. – subcapsular.

Toxicokinetics

Blood samples were taken in Weeks 13, 26 and 52 at 24 hours post-dose.

At Weeks 13, 26 and 52, M and F had similar plasma exposure to FTY720 24 hours post-dose; plasma exposure generally increased dose-proportionally between LD and MD, but was greater than dose-proportionally between MD and HD.

Table of FTY720 concentrations 24 hours post-administration (Sponsor's)

Dose level (mg/kg/day)	C_{24} (ng/ml) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	Week 52 Males	Week 52 Females
1	21.0	22.9	30.4	29.5	27.7	27.3
	(7.4)	(5.8)	(5.1)	(2.2)	(9.1)	(4.9)
3	58.2	109.6	61.9	84.3	68.8	65.8
	(17.2)	(41.8)	(9.3)	(38.0)	(26.2)	(22.6)
10	329.2	281.5	228.0	310.5	239.9	271.6
	(58.9)	(110.0)	(47.9)	(88.9)	(104.9)	(81.1)

Standard deviations are in parenthesis.

Stability and Homogeneity

Samples were taken from all dose group solutions at Weeks 1, 13, 26, 39 and 52. After analysis, data ranged from 94.6-103.2% from the target concentration. FTY720 was chemically stable for at least 15 days at 4°C and for 2 days at room temperature. Samples were taken from solutions of 0.25 and 10 mg/ml at different time points that included; initial, 4 hours and 2 days at room temperature, 2, 8 and 15 Days at 4°C. Stability data showed that the 0.25 and 10 mg/ml solutions ranged between 94.8-101% of the target concentration after 4 hours at room temperature and ranged between 91.6-99.7% of the target concentration after 15 days at 4°C.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Reverse mutation test of Y-32720 using bacteria

Study no.:	PCS-r6882
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2 February 1994
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Y-32720, Lot № I, 100.0%

Key Study Findings:

- Inhibition of growth occurred above 100 µg and there was no increase in revertant colonies observed in any strain.
- FTY720 is negative for reverse mutations and the Sponsor's conclusion that "*Y-32720 had no potential for inducing reverse mutation*" appears appropriate.
- However, 2-aminoanthracene was the sole positive control for S-9 mix, with no further characterization of the batch performed.

Methods

Strains:	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* WP2*uvrA*.
Concentrations in definitive study:	For *S. typhimurium* TA1535, TA1537, TA98 and TA100 doses were 1.563, 3.125, 6.25, 12.5, 25, 50 and 100 µg per plate For *E. coli* WP2*uvrA* doses were 31.25, 62.5, 125, 250, 500 and 1,000 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Doses were selected form a preliminary study at a dose range of 1-5,000 µg per plate.
Negative control:	Sterile deionized water was used as a negative control.
Positive controls:	
Without S-9 mix:	0.01 µg 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide on TA100 and WP2*uvrA*. 0.1 µg AF-2 on TA98. 80 µg 9-aminoacridine hydrochloride on TA1537 and 0.5 sodium azide on TA1535.
With S-9 mix:	0.5 µg 2-aminoanthracene (2AA) on TA98, 1 µg 2AA on TA100, 2 µg 2AA on TA1535 and TA1537 and 20 µg 2AA on WP2*uvrA*.
Incubation & sampling time:	Revertant colonies were counted visually following 48 hours of incubation at 37°C.

Study Validity

2AA was used as the positive control for all strains in the presence of S-9 mix, prepared from M SD rats treated with phenobarbital and 5,6-benzoflavone, and no data were presented to demonstrate that each batch of S-9 mix was characterized with a microsomal enzyme. Apart from the lack of a second positive control for the S-9 mix part of the study the study appears valid. Duplicates were performed for the preliminary study and triplicates for the main study; the criterion for a positive result was that FTY720 would produce a mean increase of revertant colonies double that observed for the control plates.

Results

Inhibition of growth was observed in all *S. typhimurium* strains at FTY720 doses of 100 µg and greater and for WP2*uvrA* at FTY720 doses of 1000 µg and greater in the presence and absence of S-9 mix. No increase in revertant colonies was observed in any strain at any dose of FTY720. Therefore, FTY720 was negative in the AMES assay and the Sponsor's conclusion that "*Y-32720 had no potential for inducing reverse mutation*" appears appropriate.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Chromosomal aberration test of Y-32720 with cultured cells

Study no.:	PCS-r6913
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2 February 1994
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Y-32720, Lot № I, 100.0%

Key Study Findings

- The study was invalid due to the lack of reporting of mitotic indices; however, for the concentrations used there was no increase in chromosomal aberrations in either the presence or absence of S-9.

Methods

Cell line:	Fibroblast cells from Chinese hamster lung
Concentrations in definitive study:	
Without S-9 mix:	0.5, 1 and 1.5 µg/ml,
With S-9 mix:	5, 10 and 15 µg/ml.
Basis of concentration selection:	50% inhibitory concentration of cell proliferation was determined and chosen as the highest dose level
Negative control:	Physiological saline
Positive control:	0.04 µg/ml Mitomycin C and 10 µg/ml Cyclophosphamide
Formulation/Vehicle:	Physiological saline
Incubation & sampling time:	24 and 48 hours incubations at 37°C in the absence of S-9 mix and 6 hour incubation with S-9 mix followed by an addition 18 hours after removal of S-9 mix

Study Validity

This was an invalid study as mitotic indices were not reported and, therefore, could not be assessed.

Results

No cells were viable at concentrations 3 µg/ml and greater, in the absence of S-9. With values at only 1 µg/ml the IC_{50} for cell proliferation was estimated as 1.5 µg/ml. In the presence of S-9, IC_{50} was estimated as 15 µg/ml. In the definitive study, no mitotic indices were reported and due to toxicity, only 1 concentration was analyzed in 1 of the 2 experiments performed in the presence of S-9. There was no increase in aberrations.

Study title:	Chromosomal aberration test of Y-32720 with cultured cells
Study no.:	PCS-r981806
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland
Date of study initiation:	27 May 1998
GLP compliance:	Yes (Switzerland 1986)
QA statement:	Yes (Dated: 12 January 1998)
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100.0%

Key Study Findings

- The study was invalid as mitotic indices did not reach the Sponsor's criteria of 30-50% or the OECD guidelines of greater than 50%; furthermore, in several experiment 3 concentrations were not analyzed (according to OECD guideline).
- There was a slight increase in chromosomal aberrations in the absence of S-9 at 0.465 and 6.3 µg/ml.
- FTY720 generally caused an increase in polyploidy compared to positive and negative controls.

Methods

Cell line:	V79 Chinese hamster cells
Concentrations in studies:	
CA4 (3 h) without –S-9 mix:	0.4, 0.8, 1.3, 2.2, 3.7, 6.3, 10.7 µg/ml,
CA4 (3 h) with +S-9 mix:	13.4, 15.8, 18.6, 21.9, 25.8, 30.3, 35.7 µg/ml.
CA5 (20 h) without –S-9 mix:	0.018, 0.034, 0.066, 0.126, 0.242, 0.465, 0.893 µg/ml
CA5 (3 h) with +S-9 mix:	17.9, 20.4, 23.4, 26.8, 30.7, 35.1, 40.2 µg/ml
CA6 (3 h) without –S-9 mix:	3.6, 4.3, 5.2, 6.2, 7.4, 8.9, 10.7 µg/ml
CA7 (20 h) without –S-9 mix:	0.2, 0.3, 0.6, 1.0, 1.7, 3.0, 5.4 µg/ml
CA7 (3 h) with +S-9 mix:	31.3, 32.6, 34, 35.4, 36.9, 38.5, 40.2 µg/ml
Basis of concentration selection:	Slides analyzed were the highest concentration used or analyzable or a concentration with a mitotic index between 30-50% of the corresponding solvent control.
Negative control:	DMSO
Positive control:	12.5 mM Ethyl methanesulphonate (EMS) and 15 µM Cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Dishes seeded with $7.5x10^5$ cells and incubated at 37°C for 3 hours (-S-9 and +S-9) and 20 hours (-S-9). Dishes incubated for 3 hours were washed and incubated for a further 17 hours after removal of S-9 mix.

Study Validity
This study was invalid, as mitotic indices did not reach the Sponsor's criteria of 30-50% or the OECD guidelines of greater than 50%; furthermore, in several experiments 3 concentrations were not analyzed, as recommended by OECD guidelines.

Results
In experiment CA4, the highest of the 3 concentrations assessed in the absence of S-9, 6.3 µg/ml, had 3% of cells with aberrations compared to the positive control of 11%; there was no effect in the presence of S-9. In both the absence and presence of S-9, the mitotic indices compared to the control mitotic index ranged from -15.2% to +15.2%, which did not reach the Sponsors criteria of 30-50% and do not reach OECD guidelines of a reduction greater than 50%.

In experiment CA5, only 2, instead of 3 concentrations, were assessed in both the absence and presence of S-9. In the absence of S-9 the lower concentration, 0.465 µg/ml, had 4% of cells with aberrations compared to the positive control of 11%, which was a statistically significant increase in aberrant chromosomes. As with experiment CA4, the mitotic indices (-19.8% to +9.3%) did not reach the Sponsor's or OECD criterion.

In experiment CA6, there was similar levels of aberrations observed in control and FTY720 treated cells; however, only 2 concentrations were analyzed and mitotic indices were -25.3% and -26.5% compared to negative control.

In experiment CA7, with incubation in the absence of S-9, FTY720 produced similar levels of aberrations in cells as observed in control cells, 3 concentrations were analyzed and the mitotic index for the highest concentration was 35.1%. However, in the presence of S-9 only 1 concentration was analyzed and had a mitotic index of 10.3%, no increase in aberrations was observed.

There was a high percentage of polyploidy cells (14.5-24%) observed in experiment CA4 with FTY720 treatment.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mouse bone marrow micronucleus test of Y-32720

Study no.:	PCS-r6926
Study report location:	4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4 April 1994
GLP compliance:	Yes (b) (4), 1982)
QA statement:	Yes (Dated: 4 November 1994)
Drug, lot #, and % purity:	Y-32720, Lot № I, 100.0%

Key Study Findings

- FTY720 did not show any clastogenic potential in mice *in vivo* up to 25 mg/kg.
- At 25 mg/kg, there was a decrease in polychromatic erythrocytes.

Methods

Doses in definitive study:	0, 6.25, 12.5 and 25 mg/kg
Frequency of dosing:	Daily (2 doses 0 and 24 hours)
Route of administration:	i.p.
Dose volume:	20 ml/kg
Formulation/Vehicle:	Saline
Species/Strain:	Mouse/ Male Crj:CD-1 (ICR)
Number/Sex/Group:	5
Satellite groups:	5 (control and 25 mg/kg)
Basis of dose selection:	Doses were selected based on the LD_{50} estimated from dose finding study, i.p. administration of 10, 100 and 1000 mg/kg and 15, 25, 40 and 60 mg/kg, LD_{50} was estimated as 50 mg/kg
Negative control:	Saline
Positive control:	1 mg/kg mitomycin C
Sampling times:	24 and 48 hours

Study Validity

The study was valid. However, the route of administration was i.p. rather than oral.

Results

There was no change in MPCEs with treatment of FTY720 at any dose level, in contrast to the increased levels in animals treated with the positive control mitomycin C. There was, however, a decrease in PCEs at 25 mg/kg (24 and 48 hours post-last dose) and a decrease at 6.25 mg/kg treatment 48 hours post-last dose. Therefore, FTY720 does not show any clastogenic potential in mice *in vivo* up to 25 mg/kg.

Table of blood concentration of FTY720 (Sponsor's)

Dose (mg/kg)	Hours after final treatment 2hours[1)]	4hours[2)]
0	-[3)]	-
25	1151.8 ± 286.49	897.5 ± 288.09

Study title: Oral bone marrow micronucleus test in rats

Study no.:	PCS-r981869
Study report location:	4.2.3.3.2
Conducting laboratory and location:	Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	4 May 1998
GLP compliance:	Yes (Switzerland, 1986)
QA statement:	Yes (Dated: 26 November 1998)
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100.0%

Key Study Findings

- FTY720 did not show any clastogenic potential up to 63 mg/kg.
- 200 mg/kg FTY720 showed a slight clastogenic potential in the first experiment; however, repeat of the treatment using several time points suggests that in rats *in vivo* FTY720 up to 200 mg/kg is not clastogenic.
- FTY720 was toxic to bone marrow at all tested doses.

Methods

Doses in definitive study:	0, 10, 63 and 200 mg/kg
Frequency of dosing:	Daily (2 doses with an interval of 24 hours)
Route of administration:	Oral
Dose volume:	8 ml/kg (5 ml/kg for positive control)
Formulation/Vehicle:	Saline
Species/Strain:	Rats/ Hans Wistar
Number/Sex/Group:	5
Satellite groups:	None
Basis of dose selection:	Doses were selected based on the results of a dose-finding study
Negative control:	Saline
Positive control:	15 mg/kg Cyclophosphamide
Sampling times:	24, 48 and 72 hours

Study Validity

The study was valid.

Results

In the dose-finding study, clinical signs of toxicity were observed in animals at the HD and included crouching, prone position, sedation, piloerection and forced breathing. One HD M died after the first administration. There was no change in MPCEs with treatment of 20 and 63 mg/kg FTY720; however, HD FTY720 and cyclophosphamide produced significant increases in MPCE frequency of 0.19% and 3.34%, respectively, compared to control frequency, 0.11%. There was a decrease in PCE frequency at all doses (32.6%, 31.1% and 27.7%) compared to control frequency (54.2%), suggesting bone marrow toxicity. In a second experiment with the same HD of FTY720, there was no significant change in MPCE frequency compared to control frequency at any

sampling point, 24, 48 and 72 hours post-last dose; frequencies for FTY720 were 0.13%, 0.16% and 0.19%, respectively, compared to control frequencies of 0.16%, 0.12% and 0.15%, respectively. PCEs were still decreased at all time points (24.7%, 20.1% and 12.2%, respectively).

Therefore, FTY720 did not show any clastogenic potential up to 63 mg/kg and although 200 mg/kg FTY720 showed a slight clastogenic potential in the first experiment, repeat of the treatment using several time points suggests that in rats *in vivo* FTY720 up to 200 mg/kg was not clastogenic. However, FTY720 was toxic to bone marrow at all doses.

7.4 Other Genetic Toxicity Studies

Study title: Mutation assay at the thymidine kinase locus of L5178Y mouse lymphoma cells.

Study no.:	PCS-R991861
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland.
Date of study initiation:	25 February 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100%.

Key Study Findings

- FTY720 was not mutagenic at the thymidine kinase locus of L5178Y mouse lymphoma cells up to 2.67 and 6 µg/ml in the absence and presence of S-9, respectively.

Methods

Cell line:	L5178Y mouse lymphoma cells
Concentrations in definitive study:	
24 hour treatment without S-9 mix:	0.53, 0.68, 0.89, 1.15 and 1.5 µg/ml,
3 hour treatment without S-9 mix:	0.79, 1.19, 1.78, 2.67 and 4 µg/ml,
3 hour treatment with S-9 mix:	1.78, 2.67, 4, 6 and 9 µg/ml and 2.73, 3.55, 4.62, 6 and 7.8 µg/ml
Basis of concentration selection:	Doses for the main study were selected on the basis of the results from the preliminary dose-ranging study (0.7, 2, 6, 18, 54 and 162 µg/ml).
Negative control:	DMSO
Positive control:	
Without S-9 mix:	Methyl methanesulfonate (MMS)
With S-9 mix:	Benzo(a)pyrene (B[a]P)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	For the main test treatment with S-9 mix was for 3 hours and sampling was performed after 48 hours incubation at 37°C.

Study Validity

S-9 mix was prepared from rats injected with Aroclor 1254 cell density in preliminary study was 5.3 x 10^5 cells/ml with 18.8 ml used (~10^7 cells). Test used duplicates and 5 concentrations. FTY720 was considered mutagenic if one or more of the mutant frequencies in the treated groups was significantly higher than the corresponding negative control; the linear trend analysis indicated dose relationship and the results were reproducible.

Results

Precipitation of FTY720 occurred at concentrations equal or greater than 153.6 µg/ml in the dose-ranging study. Mutant frequencies were not greatly increased in the presence or absence of S-9 and after 3 or 24 h treatment. In contrast, there was a substantial increase in mutant frequency after treatment with positive control. Therefore, FTY720 was not mutagenic at the thymidine kinase locus of L5178Y mouse lymphoma cells up to 2.67 and 6 µg/ml in the absence and presence of S-9, respectively.

8 Carcinogenicity

Background

The Sponsor submitted the final report for the 2-year mouse and rat carcinogenicity studies (study №s: PCS-r001039 and PCS-r001040) in 17 March 2005 to IND 57,293 (DAIDP; withdrawn 19 Feb 2008). At that time the reviewer (Terry Peters D.V.M.) had the following comments "*…identified inconsistencies (e.g., the number of tumors throughout the submission, the number of animals with autolytic tissues, and inconsistent diagnoses throughout the submission), which cannot be resolved with the individual line listings provided, and are not specific to any tissue*". This information was conveyed to the Sponsor via a teleconference on 12 May 2005 (see Memorandum of

telecon: IND 57,293, 29 Nov 2005). The Division requested that the histological slides be re-examined and offered two options (quoted below):

1. *Submission of the actual glass slides to the Agency, at which time the Agency will conduct its review in compliance with Good Laboratory Practice regulations (GLPs)*
2. *Independent (external) peer review of slides from both studies conducted by a pathologist, which includes re-evaluation of all tissues of all animals in both studies. The Sponsor should also provide a complete and total accounting of findings and how they differ from those listed in the original study reports. The sponsor may use a separate pathologist for each study but not a separate pathologist for female and male tissues within a study.*

On 10 Oct 2005, the Sponsor submitted questions regarding the carcinogenicity studies, to which the Division responded (16 Nov 2005) as follows:

1. Does FDA agree with the following list of tissues to be peered reviewed? Yes or No
– all tumors and all hyperplastic lesions reported
– all target tissues identified as such in the rat and mouse final study reports (see lists below)

Yes. The FDA agrees that all previously reported tumors and preneoplastic/ hyperplastic lesions from all animals should be reviewed, and all the target tissues in all the animals in the mouse and rat final carcinogenicity study reports (001039 and 001040, respectively) should be reviewed.

2. Does FDA agree with the proposal to review all tissues regardless of finding, in 10% of all animals? Yes or No

No. The FDA disagrees that a review of 10% of all tissues in all animals is adequate. As we stated in our facsimile correspondence dated July 13, 2005, at least 20% of all tissues of all animals from both studies should be reviewed.

3. Does the FDA agree with the following list of target tissues for non-neoplastic lesions in both mouse and rat studies? Yes or No
– Mouse Carcinogenicity study 001039

- *lymphatic organs: thymus, spleen, axillary and mesenteric lymph nodes*
- *lung*
- *kidneys*
- *bone marrow*

– Rat Carcinogenicity study 001040

- *lymphatic organs: thymus, spleen, axillary and mesenteric lymph nodes*
- *kidneys*
- *vascular system (systemic): most relevant were mesenteric/pancreatic vessels, heart*
- *brain*
- *lung*
- *liver*

No. The FDA does not completely agree with the list of target tissues for nonneoplastic lesions in the mouse and rat carcinogenicity studies. Please add ocular tissues and liver to the list of target tissues for the Mouse Carcinogenicity Study (001039). Otherwise, FDA agrees with the lists of target tissues for the mouse and rat carcinogenicity studies.

The basis for agreeing to peer-review of only 20% of tissues rather than "*all tissues from all animals in both studies*" is unclear.

Amended study reports for the mouse and rat carcinogenicity studies were submitted in the original NDA; electronic data sets were not included, but were submitted on 20 Jan 2010 (SDN 0005). For both studies the peer review of the histopathological findings was performed by an external pathologist (b) (4) between 4 Sep 2006 and 25 Jan 2007 for the mouse study and 20 Feb 2006 and 24 Jan 2007 for the rat study, in a manner that was consistent with the prior agreement with the Agency.

Study title: 104-week oral (gavage) carcinogenicity study in mice

Study no.:	PCS-r001039-02
Study report location:	4.2.3.4.1.1.
Conducting laboratory and location:	Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	17 April 2000
GLP compliance:	Yes (Switzerland, 2000)
QA statement:	Yes (Dated: 7 Dec 2004)
Drug, lot #, and % purity:	FTY720, Batch 9923014, 100%
CAC concurrence:	Yes on 8 Feb 2000

Protocol recommendations

The protocol for the mouse study was submitted to IND 57,293 on 23 December 1999 and was reviewed by the Exec CAC on 8 February 2000. The Exec CAC concurred with the Sponsor's proposed doses for the mouse study, but disagreed with the Sponsor's proposal that the mouse study only be conducted for 78 Weeks; they recommended a 104 Week period.

Key Study Findings

- There was a high incidence of autolysis described as either severe or as partial autolysis (males) or causing limited histological examination (females).
- There was a statistically significant increase in the incidence of malignant lymphomas in MD and HD females and males, according to the Sponsor.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study appears to be inadequate.

- There are high levels of autolysis (18% of females).
- Data were frequently miscoded in the dataset.

Appropriateness of Test Models
The mouse is an appropriate model to test for carcinogenicity over a 2 year period. In mouse pharmacokinetic and toxicology studies, major circulating metabolites observed in humans (FTY720-P, M3, M29 and M30) were also observed, although metabolite M2, M29 and M30 levels were demonstrated to be low compared to that in humans.

Evaluation of Tumor Findings
A statistical increase in incidence of malignant lymphoma was observed in MD and HD males and females. An increased incidence in myeloid leukemia was observed in MD males; however, no HD males developed myeloid leukemia and an increased incidence in hemangiomas and hemangiosarcomas was seen in HD females and MD and HD males, but was not statistical.

Methods

Doses:	0, 0, 0.025, 0.25 and 2.5 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral (gavage)
Formulation/vehicle:	(b) (4) graded water
Basis of dose selection:	Recommendation from the executive CAC *"the committee concurred with the sponsor's doses of 2.5, 0.25, and 0.025 mg/kg based on an appearance of dose limiting secondary effects, that affected survival. The 78-week study duration for mice is unacceptable. The standard mouse carcinogenicity study has a duration of 2 years."*
Species/strain:	Mice Crl:CD-1 (ICR)
Number/sex/group	
Main groups:	60
Toxicokinetic groups:	12
Age:	6-7 weeks of age at study initiation
Weight:	19.6-40.8 g
Animal housing:	Males housed individually, females in groups of 4/cage
Paradigm for dietary restriction:	N/a
Dual control employed:	Yes; both received vehicle
Interim sacrifice:	None
Deviation from study protocol:	Treatment was stopped for HD males in Week 92 due to a reduced survival The study pathologist changed in January 2002 as did the peer review pathologist.
Stability and homogeneity:	There was some variability in homogeneity (-1.7% to +7.1%) for 0.025 mg/kg and 5.9% to 11.3% for 2.5 mg/kg solutions. Stability ranged from -4.9% to 3.5% at room temperature and -8.7% to -3.6% when refrigerated.

Observations and Results

Mortality

Observations were done daily until the first mortality and twice daily thereafter.

Table of mortality incidences before terminal sacrifice in Week 104 (Sponsor's)

	Controls 1	0.025 mg/kg	0.25 mg/kg	2.5 mg/kg	Controls 2
Males (n)	30	27	36	46*	33
Females (n)	33	31	40	38	23

* treatment was stopped in week 92.

Table of terminal sacrifice for males and females (Sponsor's)

	MALE					FEMALE				
DOSE GROUP:	01	02	03	04	05	01	02	03	04	05
NO.ANIMALS:	60	60	60	60	60	60	60	60	60	60
CAUSE DEATH/MORIBUND :	60	60	60	60	60	60	60	60	60	60
– Terminal Sacrifice :	30	32	24	14	26	25	29	19	22	35
– Accidental Death :	–	1	1	1	1	1	–	1	–	2

There was a decrease in survival rate for MD and HD males and females. Survival and sacrifice data presented by the Sponsor do not appear to correlate. These include anomalies for MD (n = 61; 36+24+1) and HD (n = 61; 46+14+1) males and female Control group 1 (n = 59; 33+25+1), numbers in parenthesis are for early mortality, terminal sacrifices and accidental death, respectively. No mention is made that any animal was replaced and histopathological tables on pages 16401, 1786-7 and 2085-6) only list 60 animals necropsied for each group.

Clinical Signs

Clinical condition was evaluated daily. Detailed exams were conducted weekly. Palpable masses were evaluated monthly until Week 82 and fortnightly thereafter.

Signs appeared to be consistent among groups and included hunched posture, circling, hypoactivity, hyperactivity and many other non-specific findings. However, altered breathing and tremors were observed in animals just before death, with altered breathing signs showing dose-dependency (see table below).

Table of breathing, recumbency and tremor incidences observed before death

	Control 1			Control 2				LD			MD			HD		
Parameters	**M**	**F**	**B**	**M**	**F**	**B**	**Ave**	**M**	**F**	**B**	**M**	**F**	**B**	**M**	**F**	**B**
Breathing																
Bradypnea	1	-	**1**	-	-	**0**	**0.5**	-	-	**0**	-	1	**1**	-	-	**0**
Dyspnea	-	1	**1**	1	-	**1**	**1**	1	-	**1**	3	3	**6**	1	5	**6**
Tachypnea	-	-	**0**	-	1	**1**	**0.5**	-	1	**1**	-	1	**1**	1	-	**1**
Labored	2	1	**3**	-	1	**1**	**2**	2	4	**6**	1	4	**5**	7	4	**11**
Deep	-	1	**1**	-	-	**0**	**0.5**	1	-	**1**	-	-	**0**	-	-	**0**
Gasping	-	-	**0**	-	-	**0**	**0**	-	-	**0**	-	-	**0**	1	-	**0**
Total:	3	3	**6**	1	2	**3**	**4.5**	4	5	**9**	4	9	**13**	10	9	**19**
Positional																
Recumbency	-	-	**0**	-	-	**0**	**0**	1	-	**1**	1	-	**1**	2	-	**2**
Lateral recumbency	1	3	**4**	-	-	**0**	**2**	-	-	**0**	-	-	**0**	1	1	**2**
Ventral recumbency	1	-	**1**	-	-	**0**	**0.5**	-	4	**4**	2	1	**3**	1	-	**1**
Total:	2	3	**5**	-	-	**0**	**2.5**	1	4	**5**	3	1	**4**	4	1	**5**
Tremors	-	-	**0**	1	-	**1**	**0.5**	2	1	**3**	3	1	**4**	2	1	**3**

M - male, F - female, B - both

The number of palpable masses was comparable across dose groups throughout the study.

Table of palpable masses for males and females (Sponsor's)

	Controls 1	0.025 mg/kg	0.25 mg/kg	2.5 mg/kg	Controls 2
Males (n)	2	2	6	3	0
Females (n)	4	5	2	2	2

Body Weights
Once pre-treatment and weekly throughout treatment.

Decreased body weight gain was observed in MD and HD males in week 1. Significant decreases in body weight gain were observed, but not consistently, in MD males from Week 16 on and in HD males from week 6 on. Total mean weight gains over the 104 week study were 37.7%, 35.7%, 37.3%, 28.1% and 29.3% for control 1, control 2, LD, MD and HD males. There were occasional significant weekly decreases in weight gain in MD and HD females from week 14. Total mean weight gains over the 104 week study were 40.0%, 44.1%, 39.1%, 33.3% and 29.1% for control 1, control 2, LD, MD and HD females. At the end of the study, there were no significant weight gain changes.

Absolute body weight (g) and comparisons to control 1 (%) in week 104 (Sponsor's)

	Controls 1	0.025 mg/kg	0.25 mg/kg	2.5 mg/kg	Controls 2
Males	41	41 (100%)	39 (95%)	39 (95%)	41
Females	34	34 (100%)	33 (97%)	31 (91%)	35

Food Consumption
Cage values were taken weekly for the first 14 weeks on study and every 4 weeks from Weeks 18-78 and then fortnightly until the end of the study. Males were housed individually and females in groups of 4.

No treatment-related differences among groups were detected.

Ophthalmoscopy
Exams were conducted on 20 animals/sex/group (one control group only) pre-treatment and during Week 12 for the control and HD animals only. During Week 52, 18 males/20 females from the LD and 20 males/18 females from the MD were examined.

No significant treatment-related findings were reported by the Sponsor. However, incidences of posterior subcapsular lens opacities were detected at Week 52. The Sponsor determined that the finding was not treatment-related, as incidences of opacities were similar in female groups and low in males. However, the incidence (0%, 0%, 5% and 19% for the respective male groups; 30%, 20%, 39% and 53% for the respective female groups) is of concern, as there appears to be dose-dependency. One of 6 female control mice had bilateral opacity compared to 2 of 4, 6 of 7 and 8 of 8 for LD, MD and HD and severity (slight to moderate) increased with dose.

Hematology

Samples were collected at study termination from all survivors. Additionally, blood and bone marrow smears were prepared on all early sacrifice animals and all survivors at necropsy. The following parameters were assessed.

Table of blood parameters (Sponsor's)

erythrocytes	mean corpuscular hemoglobin	thrombocytes
hematocrit	mean corpuscular hemoglobin concentration	white blood cell count**
hemoglobin	mean corpuscular volume	red cell distribution width
inclusion bodies*	reticulocytes	

Decreased lymphocyte counts were observed in all treated animals in a dose-dependent manner. At necropsy, lymphocyte counts were higher in HD males than in LD and MD males; however, this may be attributed to stopping the treatment of the HD males during Week 92.

Gross Pathology

Macroscopic examination occurred at necropsy, on completion of 104 week treatment for surviving animals, at the time of euthanasia for moribund animals and as soon as possible after spontaneous death.

In the HD animals, poor body condition was noted with enlarged lymph nodes and thymus, small thymus was also noted. These findings were attributed to the increased incidence of malignant lymphoma in this dose group.

Gross necropsy findings in mice treated with FTY720 for up to 104 weeks (Sponsor's)

	MALE					FEMALE				
Dose (mg/kg/d)	**C 1**	**C 2**	**0.025**	**0.25**	**2.5#**	**C 1**	**C 2**	**0.025**	**0.25**	**2.5**
Enlarged Lymph nodes										
Axillary:	2	0	1	1	4					
Mesenteric:	0	0	1	0	5	4	4	2	12	15
Various:						9	9	5	16	13
Enlarged spleen						10	12	9	20	21
Thymus										
Enlarged:	0	0	1	0	3					
Small:	4	5	3	5	9					
Enlarged liver						0	2	0	6	8

 # Dosing stopped Week 92.

Histopathology

The tissues listed in the table below were taken from all animals; however, some unusual procedures were followed. The eyes with optic nerves, Harderian glands, lacrimal glands, pituitary and Zymbal's glands were preserved *in situ* (in the skull). Tissue processing for premature decedents, the second control group and the satellite PK animals was done "in house" while the "wet tissues" from all other animals were sent to a contract laboratory [redacted] (b) (4) for processing.

Table of tissue for histological assessment (Sponsor's)

adrenals	knee joint (femur/tibia)	seminal vesicles
aorta	lacrimal glands*	skeletal muscle
bone marrow (in bone)	liver	skin
brain	lungs	spinal cord
cecum	lymph node: axillar	spleen
clitoral gland	lymph node: mesenteric	sternum
colon	lymph node: mandibular	stomach
duodenum	mammary area	testes
epididymides	nasal cavity (passage)	thymus
esophagus	ovaries	thyroid with parathyroids**
eyes with optic nerves*	pancreas	tongue
gall bladder	peripheral nerves (sciatic)	trachea
Harderian glands*	pituitary*	urinary bladder
heart	preputial gland	uterus (with cervix)
ileum	prostate	vagina
jejunum	rectum	zymbal's glands*
kidneys	salivary glands	

and all other organs/tissues showing macroscopic abnormalities (including palpable masses) during necropsy and animal identification (implanted microchip).

* Were not separated but fixed within the skull.

** Taken with trachea.

Peer Review: Yes, but both the study pathologist and peer review pathologist changed during the 20th month on study. The males were evaluated by Dr. (b) (4) and the females by (b) (4), who replaced Dr. (b) (4) 30 Apr 2001. The study peer reviewer was (b) (4) (b) (4) who was replaced by (b) (4), and (b) (4) signed as reviewer. The results presented in the report reflect the mutually agreed-on diagnoses. Toxicokinetic animals were examined grossly, but only macroscopic abnormalities were evaluated histologically. Immunohistochemical staining for B- and T-lymphocytes was conducted on animals (10/sex from controls and HD only) with malignant lymphoma.

Observations of autolysis (Sponsor's)

	MALE					FEMALE				
DOSE GROUP:	01	02	03	04	05	01	02	03	04	05
NO.ANIMALS:	60	60	60	60	60	60	60	60	60	60
GENERAL OBSERVATIONS :	—	—	—	1	—	13	12	9	14	5
— Autolysis :	—	—	—	1	—	13	12	9	14	5

The Sponsor reported the incidence of autolysis as low in males (1), but high in females (53) (see table above); however, the incidences of partial autolysis, especially in males, were much higher. The number of incidences for animals recorded with some autolysis (severe or partial), based on the line listings were 9, 14, 17, 12 and 10 males and 15, 16, 10, 15 and 6 females in control 1, LD MD, HD and control 2 groups, respectively, for a total of 21% of animals. Of these animals 38 males and 39 females had equal or greater than 10 tissues (~20% of tissues) displaying some autolysis, causing a limited

histological examination. Thus, this large numbers of animals with autolysis prevents a definitive determination of histopathology findings.

Neoplastic: The Sponsor described an increase in malignant lymphoma in the MD and HD males and females. The Sponsor stated that the high incidence in the HD males was "*related to an overdosage consistent with the high mortality rate*" and also stated that the increased incidence was due to the pharmacological activity. No differences between the cell types of the malignant lymphoma in controls and treated animals were observed after immunohistochemical evaluation.

Males: An increase in incidence of malignant lymphoma across dose groups for MD and HD was found. Five MD males were had myeloid leukemia; this incidence was increased compared to control animals. No HD males developed myeloid leukemia, but only 21 of 60 animals survived to week 92. A dose-dependent increase in incidence of hemangiomas and hemangiosarcomas was also observed in MD and HD males.

Females: There was an increase in malignant lymphoma in MD and HD females and of hemangiomas and hemangiosarcomas in HD females.

Table of observed Neoplasms after FTY720 treatment for up to 104 Weeks

	MALE					FEMALE				
Neoplasms	**0**	**0**	**0.025**	**0.25**	**2.5**	**0**	**0**	**0.025**	**0.25**	**2.5**
Malignant lymphoma	4	8	5	11	18	15	21	17	26	26
Histiocytic sarcoma	1	0	0	1	3	2	3	3	2	2
Myeloid leukemia	0	1	2	5	0	0	0	0	0	0
Erythroid leukemia	0	0	0	0	0	1	0	0	0	0
Malignant fib. histiocytoma	0	0	1	0	0	1	0	1	1	0
All Systemic	5	9	8	17	21	19	24	20	28	28
Hemangioma	0	0	0	0	0	1	0	2	0	4
Hemangiosarcoma	2	1	1	3	6	2	0	1	3	2
Hemangioma + -sarcoma	2	1	1	3	6	3	0	3	3	6
Liver cholangioma	0	0	0	0	0	0	0	1	1	0
Duodenum adenocarcinoma	0	0	0	0	1	0	1	0	0	0
Rectum adenocarcinoma	0	0	0	0	0	0	0	0	1	0
Spinal cord paraganglioma	0	0	0	1	0	0	0	0	0	0
Spinal cord sarcoma metastasis	0	0	0	0	0	0	0	0	1	0
Eyes carcinoma metastasis	0	0	0	0	0	0	0	0	1	0
Knee joint chrondroma	0	0	0	1	0	0	0	0	0	0

Non-neoplastic: Target organs for FTY720 defined by the Sponsor were the thymus, spleen, axillary and mesenteric lymph nodes, lungs, kidneys and bone marrow. Observations seen in the lymphoid tissues (thymus, spleen, lymph nodes) appeared to be consistent with the pharmacological action of FTY720, however there was a decrease in lymphocytes in the lymph nodes, in an apparent contradiction to the mode of action, prevention of egress of lymphocytes from lymphoid organs. Findings included

thymic, thymic architecture modification (cortical atrophy), lymphoid atrophy, medullary enlargement of the thymus, lymphoid atrophy in the periarteriolar lymphatic sheaths in the spleen and decreased numbers of lymphocytes in lymph nodes. Other findings included increased renal changes (tubular, intranuclear inclusion bodies) in MD and HD females. In these same animals, increased "follicle-like" lymphoid aggregates were observed in bone marrow. These findings appear treatment-related, as there was a dose-dependent increase in incidence and severity. The Sponsor suggested that these findings may represent an exacerbation of background lesions, but does not preclude a treatment effect.

A dose-dependent increase in the incidence and severity of amyloidosis, alveolar macrophages, fibrosis and osseous metaplasia was found in the lungs of MD and HD animals and acidophilic pneumonia in MD and HD females. Furthermore, the Sponsor put forward that the increased severity of amyloidosis coupled with the increased incidence of nephropathy in females were the main reasons for increased morbidity and mortality in the MD and HD groups. The Sponsor also commented that "*any interference with the immune system of mice favors the development of amyloidosis and has an impact on the type of amyloidosis.*"

Relevant non-neoplastic findings after FTY720 treatment for up to 104 Weeks

	MALE						FEMALE					
Group (mg/kg/day)	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5[#]**	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5**
Spleen	(59)	(60)		(60)	(60)	(60)	(60)	(59)		(59)	(60)	(60)
Amyloidosis:	13	13	**13**	9	5	7	11	5	**8**	12	8	6
Lymphoid atrophy:	6	5	**5.5**	9	19	30	11	9	**10**	12	12	18
Increased hematopoiesis:	17	14	**15.5**	22	27	26	17	13	**15**	21	18	29
Thymus	(44)	(47)		(43)	(52)	(43)	(51)	(54)		(51)	(45)	(47)
Tinctorial change:	0	0	**0**	0	16	11	0	0	**0**	0	0	0
Enlarged medulla:	0	0	**0**	0	16	11	0	0	**0**	0	0	0
Cortical atrophy:	0	2	**1**	1	15	11						
Mesenteric L. Node	(55)	(54)		(49)	(57)	(54)	(54)	(55)		(53)	(53)	(56)
Lymphoid atrophy:	14	14	**14**	3	19	19	15	14	**14.5**	22	19	24
Arteritis:	0	0	**0**	1	0	1	1	5	**3**	2	5	9
Sinusoids depletion:	5	2	**3.5**	0	15	14	0	0	**0**	0	0	0
Mandibular L. Node	(46)	(46)		(46)	(49)	(52)	(51)	(54)		(57)	(51)	(50)
Granulocyte infiltration:	0	0	**0**	0	0	0	1	5	**3**	2	5	9
Axillary L. Node	(51)	(50)		(53)	(48)	(52)	(58)	(48)		(50)	(54)	(47)
Lymphoid atrophy:	0	0	**0**	0	1	5	1	0	**5**	0	1	0
Lungs	(60)	(60)		(60)	(60)	(60)	(60)	(60)		(60)	(60)	(60)
Amyloidosis:	9	14	**11.5**	9	17	16	8	3	**5.5**	5	16	18
Fibrosis:	0	0	**0**	0	1	3	2	2	**2**	3	5	2
Alveolar macrophages:	1	2	**1.5**	4	11	20	7	8	**7.5**	10	16	30
Osseous metaplasia:	1	4	**2.5**	0	6	11	0	1	**0.5**	0	1	5
Acidophilic pneumonia:	0	0	**0**	4	1	0	2	5	**3.5**	2	6	10
Kidney	(60)	(60)		(60)	(60)	(60)	(60)	(60)		(60)	(60)	(60)
Nephropathy:	38	45	**41.5**	42	50	42	27	25	**26**	28	37	43
Intranuclear inclusions:	0	0	**0**	0	0	0	0	0	**0**	0	17	23
Liver	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)		(60)	(60)	(60)
Hematopoiesis:	1	0	**0.5**	0	0	4	2	2	**2**	2	5	1
Bile duct hyperplasia:	0	0	**0**	0	0	0	1	1	**0**	0	0	0

	MALE						FEMALE					
Group (mg/kg/day)	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5#**	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5**
Pancreas	(60)	(54)		(59)	(60)	(59)	(58)	(59)		(59)	(59)	(57)
Arteritis:	3	3	**3**	0	0	1	3	2	**2.5**	1	2	7
Edema:	0	1	**0.5**	0	0	1	0	3	**1.5**	1	4	9
Cecum	(56)	(52)		(50)	(48)	(54)	(51)	(56)		(50)	(54)	(57)
Arteritis:	0	0	**0**	0	0	0	3	3	**3**	2	6	6
Edema:	3	0	**1.5**	1	0	0	0	1	**0.5**	2	4	5
Eyes	(60)	(59)		(60)	(60)	(59)	(59)	(60)		(57)	(58)	(59)
Inflammation:	0	0	**0**	0	1	4	2	6	**4**	8	9	3
Harderian gland	(59)	(59)		(60)	(60)	(59)	(60)	(60)		(58)	(59)	(59)
Atrophy:	4	2	**3**	0	1	7	1	1	**1**	2	0	1
Lacrimal gland	(55)	(58)		(56)	(58)	(54)	(56)	(60)		(54)	(55)	(55)
Atrophy:	8	5	**6.5**	2	0	8	6	2	**4**	0	5	2
Nasal cavity	(60)	(60)		(60)	(60)	(59)	(60)	(60)		(58)	(60)	(60)
Inflammation:	2	3	**2.5**	10	3	11	6	3	**4.5**	0	3	3
Zymbal's gland	(30)	(5)		(32)	(47)	(22)	(28)	(22)		(47)	(45)	(31)
Inflammation:	0	0	**0**	0	0	3	3	1	**2**	3	2	1
Bone marrow (sternum)	(59)	(59)		(60)	(60)	(59)	(60)	(59)	(60)	(60)	(60)	(58)
Hypercellularity:	4	7	**5.5**	3	1	10	12	11	**11.5**	12	12	9
Lymphoid follicles:	0	0	**0**	0	7	1	5	7	**6**	8	18	20
Bone marrow (femur)	(60)	(60)	(60)	(60)	(60)	(58)	(59)	(59)		(57)	(60)	(60)
Hypercellularity:	5	7	**6**	3	1	11	11	11	**11**	11	11	8
Hypocellularity:	2	1	**1.5**	0	0	0	20	16	**18**	13	6	7
Lymphoid follicles:	1	1	**1**	0	3	1	8	9	**8.5**	6	18	17
Bone marrow (cord)							(60)	(60)		(60)	(60)	(60)
Lymphoid follicles:							0	5	**2.5**	2	11	19
Seminal vesicles	(60)	(60)		(60)	(60)	(60)						
Decreased secretion:	2	3	**2.5**	1	1	8	-	-	**-**	-	-	-
Inspissated secretion:	2	2	**2**	0	3	7	-	-	**-**	-	-	-
Uterus							(60)	(60)		(58)	(59)	(58)
Arteritis:	-	-	**-**	-	-	-	6	10	**8**	12	10	18

 # Treatment stopped Week 92.

Toxicokinetics

Samples were taken during Weeks 10, 26 and 52 from 3 animals/sex at 1, 4, 7 and 24 hours post-dose.

The Sponsor stated that FTY720 was not found in any control sample. Plasma exposure appeared dose-proportional with exposure being greater in males than female. Accumulation was apparent over the course of the study.

Table of toxicokinetic parameters for FTY720 in males (Sponsor's)

	0.025 mg/kg/day			0.25 mg/kg/day			2.5 mg/kg/day		
	Wk 10	Wk 26	Wk 52	Wk 10	Wk 26	Wk 52	Wk 10	Wk 26	Wk 52
t_{max}	7	7	7	4	4	4	4	4	4
C_{max}	1.313	2.472	1.956	16.850	16.198	21.629	189.566	175.937	226.697
C_{max}/dose	52.5	98.9	78.2	67.4	64.8	86.5	75.8	70.4	90.7
AUC(0-24h)	24.8	46.9	39.7	285.0	277.0	360.0	3318.0	3210.0	4211.0
AUC(0-24h)/dose	992	1874	1589	1139	1107	1440	1327	1284	1685

t_{max} is expressed in h. C_{max} is expressed in ng/mL. AUC(0-24h) is expressed in ng·h/mL. AUC(0-24h)/dose is expressed in (ng·h/mL)/(mg/kg/day). For calculation of AUC(0-24h), the concentration at 0 was replaced by the concentration measured at 24h.

Table of toxicokinetic parameters for FTY720 in females (Sponsor's)

	0.025 mg/kg/day			0.25 mg/kg/day			2.5 mg/kg/day		
	Wk 10	Wk 26	Wk 52	Wk 10	Wk 26	Wk 52	Wk 10	Wk 26	Wk 52
t_{max}	7	4	7	7	7	7	7	4	7
C_{max}	0.818	1.597	1.276	12.067	13.114	20.179	127.977	123.184	181.356
C_{max}/dose	32.7	63.9	51.0	48.3	52.5	80.7	51.2	49.3	72.5
AUC(0-24h)	18.8	31.4	25.9	223.0	233.0	351.0	2518.0	2195.0	3548.0
AUC(0-24h)/dose	752	1254	1036	893	934	1404	1007	878	1419

t_{max} is expressed in h. C_{max} is expressed in ng/mL. AUC(0-24h) is expressed in ng·h/mL. AUC(0-24h)/dose is expressed in (ng·h/mL)/(mg/kg/day). For calculation of AUC(0-24h), the concentration at 0 was replaced by the concentration measured at 24h.

Second Peer review

A second full peer review was requested by the Agency. The review was performed by an external pathologist [redacted] (b) (4) between 4 Sep 2006 and 25 Jan 2007, and was consistent with the Agency's request. The peer review included a review of the histological slides from all tumors and pre-neoplastic findings (focal hyperplasias), slides from thymus, spleen, axillary and mesenteric lymph nodes, lung, kidneys, bone marrow, eyes and liver from all animals, and all organs/tissues from 120 animals.

Based on the results of the peer review, the Sponsor concluded that there were only commonly occurring tumors found in this study and that the incidence, distribution and morphologic appearance gave no "*indication of a direct compound-related occurrence.*" However, as there is a treatment related increase in tumors, albeit possibly through an exaggerated pharmacology the relevance of these observations should not be diminished.

There was no change in the reporting of autolysis; thus, the high incidence rate remains, with the large numbers of animals with autolysis, severe or partial, making at least determination of histopathology findings difficult and possible obscuring.

Neoplastic: There were changes in malignant lymphoma reporting, however reporting of changes was confusing

Eight (637, 640, 643, 647, 662, 667, 677 and 683) control group 2 females had the original finding deleted and female 693 maintained the finding but with a grade of N4. However, in the table (pg 40) labeled "*Number of Animals with Neoplastic Lesions by Organ/group/sex Males K0 Incl. Deaths*" the number of malignant lymphomas was reduced by 7 and not 8.

Animal 596 change was placed in group 4 instead of group 5 (page 53) that resulted in the wrong number of adenoma's reported.

Other changes to the pathology report can be observed in the table below.

Males: A statistically significant increase was found in the incidence of malignant lymphoma across dose groups (p-value of < 0.001) with pairwise comparisons showing significance for MD (p-value of 0.0393) and HD (p-value of < 0.001) groups. One incidence of myeloid leukemia in the MD group was deleted leaving 4 incidences; this incidence was significantly increased compared to control animals (p-value of 0.0382). No HD males developed myeloid leukemia, but only 21 of 60 animals survived to 92 weeks. The increased incidence in hemangiomas and hemangiosarcomas observed in MD and HD males was maintained, but was not statistical.

Females: The only statistically significant increase in tumors was for malignant lymphoma in MD (p = 0.0047, poly-3 pairwise) and HD (p = 0.0177) females. There was an increase in hemangiomas and hemangiosarcomas in HD females, but the effect was not statistically significant.

Table of changes to observed Neoplasms from original and peer review

	MALE					FEMALE				
Neoplasms	**0**	**0**	**0.025**	**0.25**	**2.5**	**0**	**0**	**0.025**	**0.25**	**2.5**
Malignant lymphoma	(4) **1**	8	5	(11) **10**	18	(15) **10**	(21) **13***	(17) **12**	(26) **25**	(26) **24**
Histiocytic sarcoma	1	0	0	(1) **0**	(3) **2**	2	(3) **2**	3	(2) **1**	(2) **1**
Myeloid leukemia	0	1	2	(5) **4**	0	0	0	0	0	0
Erythroid leukemia	0	0	0	0	0	1	0	0	0	0
Malignant fib. histiocytoma	0	0	1	0	0	1	0	1	1	0
All Systemic	(5) **2**	9	8	(17) **14**	(21) **20**	(19) **14**	(24) **16**	(20) **15**	(28) **26**	(28) **25**
Hemangioma	(0) **1**	0	0	0	0	1	0	2	0	(4) **5**
Hemangiosarcoma	(2) **1**	1	1	3	6	2	0	1	3	(2) **1**
Hemangioma + -sarcoma	2	1	1	3	6	3	0	3	3	6
Lung adenoma	(13) **12**	11	10	9	4	9	(10) **11**	(12) **11**	9	10
Lung carcinoma	0	0	0	0	0	3	10	5	6	(4) **5**
Heart carcinoma metastasis	0	0	0	0	0	0	(0) **1**	(1) **0**	0	(0) **1**
Liver adenoma	(4) **6**	(5) **6***	(3) **5**	(3) **2**	1	0	0	(0) **1**	(0) **1**	0
Liver carcinoma	(5) **2**	(7) **5**	(3) **1**	3	2	0	0	1	(2) **1**	0

	MALE					FEMALE				
Neoplasms	**0**	**0**	**0.025**	**0.25**	**2.5**	**0**	**0**	**0.025**	**0.25**	**2.5**
Liver cholangioma	0	0	0	0	0	0	0	1	(1) **0**	0
Gall bladder adenoma	0	0	0	0	0	(1) **0**	0	0	0	0
Salivary sarcoma metastasis	0	0	0	0	0	0	0	(1) **0**	0	0
Stomach adenoma	1	0	(1) **0**	0	0	0	(0) **1**	0	0	0
Stomach carcinoma	(1) **0**	0	0	0	0	0	(1) **0**	0	0	0
Duodenum adenoma	0	0	0	0	(0) **1**	0	(0) **1**	0	0	0
Duodenum adenocarcinoma	0	0	0	0	(1) **0**	0	(1) **0**	0	0	0
Jejunum adenocarcinoma	0	(0) **1**	0	0	0	0	0	0	0	0
Cecum adenocarcinoma	(0) **1**	0	0	0	0	0	0	0	0	0
Rectum adenocarcinoma	0	0	0	0	0	0	0	0	(1) **0**	0
Thymus sarcoma metastasis	0	0	0	0	0	0	1	0	(1) **0**	0
LN carcinoma metastasis	0	0	0	0	0	0	0	(1) **0**	0	0
Pancreas adenoma	0	0	0	0	0	0	(1) **0**	0	0	0
Kidneys adenoma	0	(0) **1**	0	0	0	0	0	0	0	0
Kidneys carcinoma	0	(1) **0**	0	0	0	0	0	0	0	0
Adrenal adenoma	(3) **2**	2	1	(1) **2**	0	0	0	0	0	0
Seminal vesicle adenoma	1	(0) **1**	1	0	0	0	0	0	0	0
Ovary cystadenoma	0	0	0	0	0	(2) **3**	2	3	2	1
Ovary cystadenocarcinoma	0	0	0	0	0	(1) **0**	0	0	0	0
Ovary luteoma	0	0	0	0	0	(1) **2**	1	(2) **0**	0	1
Ovary tumor	0	0	0	0	0	(1) **0**	1	0	0	0
Uterus adenoma	0	0	0	0	0	(1) **0**	0	0	0	0
Uterus adenocarcinoma	0	0	0	0	0	(1) **0**	0	0	0	0
Uterus leiomyoma	0	0	0	0	0	1	2	1	2	(1) **0**
Uterus tumor	0	0	0	0	0	0	2	(1) **0**	(2) **1**	1
Vagina fibroma	0	0	0	0	0	0	0	(0) **1**	0	0
Vagina fibrosarcoma	0	0	0	0	0	0	0	(1) **0**	0	0
Spinal cord paraganglioma	0	0	0	(1) **0**	0	0	0	0	0	0
Spinal cord meningioma	0	0	0	(0) **1**	0	0	0	0	0	0
Spinal cord sarcoma metastasis	0	0	0	0	0	0	0	0	(1) **0**	0
Eyes carcinoma metastasis	0	0	0	0	0	0	0	0	(1) **0**	0
Harderian gland adenoma	(5) **6**	3	4	(1) **2**	2	(2) **1**	2	(4) **3**	2	(2) **1**
Skin basal carcinoma	(1) **0**	0	0	(1) **0**	0	0	0	0	0	0
Skin squamous carcinoma	0	0	0	(0) **1**	0	0	0	0	0	0
Skin carcinoma metastasis	0	0	0	0	0	0	0	(1) **0**	0	0
Skin tumor	0	0	0	0	0	(1) **0**	0	0	0	0
Knee joint chrondroma	0	0	0	(1) **0**	0	0	0	0	0	0

Initial incidences in parenthesis, changed values after peer review in bold. * Inconstancies.

Non-neoplastic: Based on the second peer review, there was an increase in the incidence of enlarged thymic medulla in HD female, an increase in axillary lymph node lymphoid atrophy and the findings of acidiophilic pneumonia in female lungs were changed to chronic inflammation. Changes in hyperplasia (see table below) and lymphoid cell infiltrations incidences also occurred in several tissue across the groups.

Changes to relevant non-neoplastic findings after Peer-review

	MALE						FEMALE					
Group (mg/kg/day)	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5#**	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5**
Thymus												
Tinctorial change:	0	0	0	0	16	11						
Enlarged medulla:	0	0	0	0	16	11	0	0	0	0	(0) **12**	(0) **12**
Cortical atrophy:	0	(2) **0**	**0**	(1) **0**	(15) **0**	(11) **0**						
Diffuse atrophy:	(20) **0**	(28) **0**	**0**	(15) **0**	(26) **0**	(17) **0**						
Atrophy:	(0) **20**	(0) **30**	**15**	(0) **16**	(0) **41**	(0) **28**	31	20	25.5	23	20	25
Axillary L. Node												
Lymphoid atrophy:	0	0	0	0	1	5	(1) **5**	(0) **5**	5	(0) **4**	(1) **3**	(0) **11**
Lungs												
Amyloidosis:	9	14	11.5	9	17	16	8	3	5.5	5	16	18
Fibrosis:	0	0	0	0	1	3	2	2	2	3	5	2
Alveolar macrophages:	1	2	1.5	4	11	20	7	8	7.5	10	16	30
Osseous metaplasia:	1	4	2.5	0	6	11	0	1	0.5	0	1	5
Acidophilic pneumonia:	0	0	0	4	1	0	(2) **0**	(5) **0**	0	(2) **0**	(6) **0**	(10) **0**
Liver												
Hematopoiesis:	1	0	0.5	0	0	4	2	2	2	2	5	1
Bile duct hyperplasia:							(1) **0**	(1) **0**	0	0	(0) **1**	0

 # Treatment stopped Week 92. Old incidences in parenthesis, changed values after peer review in bold.

Changes in hyperplasia incidences

		MALE						FEMALE					
Group (mg/kg/day)		**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5#**	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5**
Lungs		(0) **1**	5	**3**	3	0	1	2	3	2.5	(2) **3**	(9) **8**	5
Spleen	Lymphoid:	(4) **7**	2	**4.5**	8	(4) **6**	(9) **10**	(13) **17**	(17) **23**	**20**	(21) **24**	(15) **17**	(9) **11**
	Myeloid:	0	0	0	0	(0) **1**	0						
Thymus	Lymphoid:	(4) **6**	(3) **4**	**5**	4	0	0	(10) **14**	(14) **19**	**14.5**	(20) **24**	(10) **11**	(8) **10**
	Atypical:	0	(2) **0**	**0**	0	0	1						
Mandibular LN		(1) **2**	0	**1**	0	(2) **4**	0	(5) **7**	(3) **4**	**5.5**	(3) **5**	(11) **13**	(5) **6**
Mesenteric LN	Lymphoid:	2	0	1	0	(1) **2**	1	(4) **6**	2	**4**	2	3	(3) **4**
	Angiomatous:	0	0	0	0	0	0	3	(2) **1**	**2**	0	0	0
Axillary LN		1	2	1.5	1	(0) **1**	0	(2) **3**	(1) **3**	**3**	4	(3) **4**	(3) **4**
Lymph nodes (LN)		(0) **1**	0	**0.5**	0	0	0	1	2	1.5	2	(3) **4**	(2) **3**
Liver	Ito cell:	1	0	0.5	0	0	0	0	0	0	0	0	0
	Oval cell:	(1) **0**	(1) **0**	**0**	0	0	0	0	0	0	0	0	0
	Bile duct:	0	0	0	0	0	0	(1) **0**	(1) **0**	**0**	0	(0) **1**	0
	Hepatocellular:	0	0	0	0	0	0	0	0	0	0	(1) **0**	1
Gall bladder		0	0	0	0	0	0	(1) **2**	2	**2**	0	0	2
Pituitary gland		0	0	0	0	0	0	(15) **0**	(15) **0**	**0**	(12) **0**	(11) **0**	(14) **0**
Kidneys		0	0	0	0	0	0	1	(3) **1**	**1**	(2) **1**	(3) **2**	1
Adrenal glands		8	10	9	8	(11) **10**	6	(2) **1**	6	**3.5**	(6) **5**	0	1
Thyroid gland		0	0	0	0	0	0	8	(8) **7**	**7.5**	9	8	6
Parathyroid gland		0	0	0	0	0	0	0	(3) **1**	**0.5**	1	2	0
Harderian gland		(3) **2**	2	**2**	1	1	1	(2) **3**	3	**3**	(6) **5**	1	4
Bone marrow (sternum)		0	0	0	0	(0) **1**	0	0	0	0	0	0	0
Bone marrow (femur)		0	0	0	0	(0) **1**	0	0	0	0	0	(1) **0**	0
Testes		(2) **1**	1	**1**	0	1	0	-	-	-	-	-	-
Mammary area	Focal:							2	(3) **1**	**1.5**	1	2	0
	Diffuse:							3	(4) **1**	**2**	(4) **3**	0	2

		MALE						FEMALE					
Group (mg/kg/day)		**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5#**	**0**	**0**	**Ave**	**0.025**	**0.25**	**2.5**
Ovary	Angiomatous:	-	-	-	-	-	-	(1) **0**	0	**0**	0	0	0
	Cystic:	-	-	-	-	-	-	1	2	1.5	(8) **5**	(2) **1**	(3) **2**
	Interstitual cell:	-	-	-	-	-	-	(1) **0**	2	**1**	3	2	1
	Stromal:	-	-	-	-	-	-	(2) **0**	(2) **1**	**1.5**	0	1	0
	Sex cord:	-	-	-	-	-	-	1	(2) **1**	**1**	4	(2) **1**	0
Uterus	Smooth muscle:	-	-	-	-	-	-	0	0	**0**	1	0	(1) **2**
	Stromal:	-	-	-	-	-	-	3	(4) **3**	**3**	0	1	2

 # Treatment stopped Week 92. Old incidences in parenthesis, changed values after peer review in bold.

Overall summary

The mouse carcinogenicity study appears to be inadequate.

- There were several inconsistencies throughout the submission including survival and sacrifice data, however this alone would not make the study inadequate,
- In the dataset organs that were reported as autolyzed to some extend appear to have been included only when at least one animal in the study had been found to have a tumor in that organ. Thus, for instance, since no tumor was found in any gall bladder, instances of gall bladder autolysis have not been recorded in the dataset. From the inaccurate dataset striking differences in the patterns are observed. In males, 11% of the total number of mice had autolyzed cecum, the colon (5%), duodenum, (6.7%) and jejunum (7%). However, with the exception of the duodenum (8%), none of these organs were diagnosed as autolyzed in female mice. This likely reflects the fact that two different pathologists assessed males and females. Autolysis reporting was not changed in the peer review. However, based on the line listings there was a high incidence of autolysis; 13% of males and females had equal or greater than 20% of organs reported as having some autolysis.
- Coding in the dataset was confusing, with it often impossible to distinguish or determine if organs were unexamined or just miscoded.
- After the second peer review there were over 300 changes, however, there were few major changes. Examples of changes include:
  - malignant lymphoma replaced by either lymphoid hyperplasia or lymphoid infiltration,
  - hepatocellular adenoma replaced by focus of cellular alteration, basophil, grade 2,
  - histiocytic sarcoma replaced by malignant lymphoma,
  - hemangioma replaced by hemorrhage, diffuse, submucosal with angiectasis, grade 4
  - duodenum, focal/segmental mucosal hyperplasia, grade 4 the finding was deleted,
  - liver, focus of cellular alteration, eosinophilic, grade 5 the finding was deleted,
  - heart, metastatic carcinoma, focal arising from lung carcinoma the finding was deleted,
  - metastatic sarcoma arising from body cavity mass the finding was deleted,

Selected doses of FTY720 produced an increased mortality of MD and HD animals compared to controls. The treatment of HD males was stopped prematurely during Week 92 due to this reduced survival rate; however, as this was only 3 months prior to terminal sacrifice, the study was not compromised. Lymphocyte counts were significantly decreased in all treated animal groups as would be expected from the pharmacological action of FTY720 and resulted in atrophy of the lymphatic organs. After a second peer-review (requested by the Agency), there were a number of changes, but these were relatively minor. A high incidence of autolysis was reported, either as severe or partial, that allowed for limited evaluation of the organs by the Sponsor.

There was a statistically significant increase in the incidence of malignant lymphoma for MD and HD groups. The Sponsor stated that the increased incidence of malignant lymphomas was due to the pharmacological activity of the test compound. This is a reasonable conclusion as pharmacologically active immunosuppressants have been shown previously to increase the incidence of malignant lymphomas (Maniszewska, Arch Immunol Ther Exp., 25, 227-233, 1977; Reyes *et al*., Diagn Cytopathol., 12, 32-36, 1995). The claim by the Sponsor that the high incidence in the HD males could be "*related to an overdosage consistent with the high mortality rate*" does not preclude the dose-dependent nature of this observation and that it is the effect of treatment. Myeloid leukemia was observed in 4 MD males; however, this unlikely to be treatment related as no incidence was observed in the HD group. Increased incidence of hemangiomas and hemangiosarcomas was also observed in MD and HD males and HD females, but was not statistically significant. Although the Sponsor did not consider the findings toxicologically significant, as the number incidences fell within historical limits, the incidences in males were dose-dependent and, therefore, a biologically relevant effect can not be precluded.
Although this study is inadequate due to excessive autolysis, a statistical dose-dependent increase in the incidence of malignant lymphoma was observed. Although the conclusion by the Sponsor is that "*Oral administration of FTY720 to mice for 104 weeks showed no evidence of a direct tumorigenic effect representing a maximal exposure (AUC0-24h) of 2195 to 4211 ng·h/ml*", the treatment related increase in malignant lymphomas, even though expected from this class of drug, should not be diminished.

Study title: 104-Week oral (gavage) carcinogenicity study in rats

Study no.:	PCS-r001040-01
Study report location:	4.2.3.4.1.1.
Conducting laboratory and location:	Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	17 May 2000
GLP compliance:	Yes (Switzerland, 2000)
QA statement:	Yes (Dated: 27 Aug 2004)
Drug, lot #, and % purity:	FTY720, Batch 9923014, presumed 100%
CAC concurrence:	Yes on 8 Feb 2000

Protocol recommendations

The protocol for the rat study was submitted to IND 57,293 on 23 December 1999 and was reviewed by the Exec CAC on 8 February 2000. The Exec CAC did not agree with the proposed doses for the rat study. Based on the limited data provided, the MTD was estimated as 2.5 mg/kg/day and the doses recommended for the 2-year study were 0.05, 0.5 and 2.5 mg/kg/day.

Key Study Findings

- There was high mortality in HD females, resulting in stopping of dosing in Week 75 and early termination, along with control group 2, in Week 95. In addition, dosing in HD males was stopped in Week 99 due to an increase in mortality.
- Increased mortality also occurred in mid high dose (MHD) males and females.
- After the second peer review, by (b) (4), the Sponsor concluded that "*Oral administration of FTY720 to rats for 104 weeks showed no tumorigenic potential up to the highest dose level of 2.5 mg/kg/day*".

Adequacy of Carcinogenicity Study

Early cessation of dosing (week 75) and termination (week 95) of HD females did not compromise the study since there were 4 dose groups and the MHD approximated an MTD. As there was a slight increase in mortality at 0.5 mg/kg with increased clinical sign incidences, decreased body weight gain and an increase in lung histopathological findings suggesting the dose was close to an MTD therefore, the study appears adequate.

Appropriateness of Test Models

The rat is an appropriate model to test for carcinogenicity over a 2 year period. In rat pharmacokinetic and toxicology studies, major circulating metabolites observed in humans (FTY720-P, M3, M29 and M30) were also observed.

Evaluation of Tumor Findings

In males, a few rare tumors (preputial gland carcinoma and malignant melanoma of the eye) were observed, but only in individual animals. In females, there was decreased incidence of pituitary adenomas and increase in proliferative findings in the uterus that appeared dose-dependent (even when considering that HD females were stopped

dosed at Week 75 and the group terminated at Week 95); however, the increase in proliferative findings was not statistically significant.

Methods

Doses:	0, 0, 0.05, 0.15, 0.5 and 2.5 mg/kg
Frequency of dosing:	Daily
Dose volume:	2 ml/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Distilled water
Basis of dose selection:	Recommendation from the Executive CAC: "*The committee did not concur with the sponsor's recommended doses. Based on mortality and other toxic signs at 10 mg/kg in the 6 month study and smooth muscle hypertrophy with signs of immunosuppression at 5 mg/kg in the 13 week study the committee estimated that the maximum tolerated dose was likely between 0.5 and 5 mg/kg. Based on the limited evidence available, they recommended the following doses: 2.5, 0.5, and 0.05 mg/kg, with a greater high dose (2.5mg/kg/day) as more closely approximating a maximum tolerated dose.*"
Species/Strain:	Rats Han:Wist, CR/WIGA
Number/Sex/Group	
Main groups:	51
Age:	6 weeks of age at initiation of dosing
Weight:	100-197 g at initiation of dosing
Animal housing:	3 animals/sex/cage
Paradigm for dietary restriction:	Not applicable
Dual control employed:	Yes; both received the vehicle
Interim sacrifice:	None
Deviation from study protocol:	Dosing was stopped in HD females during Week 75 and HD males at Week 99 due to excessive mortality. One control group was terminated with the HD females during Week 95 when there were only 15 survivors. The 2 control groups were pooled for all statistical evaluations.
Stability and Homogeneity:	There was some variability in homogeneity; 5.5-13.8% for 0.05 mg/kg and -1.5% to 12.0% for 2.5 mg/kg solutions. Stability ranged from -5.2% to -0.8% at room temperature and -11.7% to -1.8% when refrigerated.

Observations and Results

Mortality

Daily observations were performed until the first death and twice daily thereafter.

Table of mortality incidences before terminal sacrifice in Week 104 (Sponsor's)

	Controls 1	0.05 mg/kg	0.15 mg/kg	0.5 mg/kg	2.5 mg/kg	Controls 2
Males	12	10	9	17	32	9
Females	17	19	15	30	36*	13*

* until week 95

HD animals experienced high levels of mortality, with 29.4% of males and 62.8% of females dead in the first 78 weeks. Dosing of HD females was stopped in Week 75 due to the high mortality rate, and the remaining animals were terminated in Week 95. Control group 2 was terminated at the same time as the HD females. The Sponsor states that for "*both males and females, the comparison of the two control groups yields a non-significant result. It is therefore legitimate to pool the two control groups and compare with the combined control group.*" This would not be possible for females as the 2 control groups were terminated at different time points. Treatment for HD males was ceased in Week 99, due to an increase in mortality rate compared to controls.

Clinical Signs

Clinical condition was evaluated at least daily. Detailed exams were conducted weekly. Palpable mass evaluation was conducted at least monthly until Month 12 and fortnightly thereafter.

Signs appeared generally comparable across groups, but 1 HD male showed nystagmus and convulsions and tremor. However, there were dose-related increases in altered breathing and recumbency in animals just before death (see table below).

Table of breathing, recumbency and tremor incidences observed before mortality

	Control 1			Control 2				LD			LMD			HMD			HD		
Parameters	M	F	B	M	F	B	Ave	M	F	B	M	F	B	M	F	B	M	F	B
Breathing																			
Dyspnea	0	2	**2**	2	0	**2**	**2.0**	1	3	**4**	2	0	**2**	4	3	**7**	1	5	**6**
Tachypnea																	0	2	**2**
Labored								0	1	**1**				1	0	**1**	0	1	**1**
Deep														0	1	**1**	1	2	**3**
Gasping																	0	4	**4**
Total	0	2	**2**	2	0	**2**	**2.0**	1	4	**5**	2	0	**2**	5	4	**9**	2	14	**16**
Positional Recumbency								0	1	**1**	1	0	**1**	0	2	**2**	1	0	**1**
Lateral recumbency																	1	0	**1**
Ventral recumbency				1	2	**3**	**1.5**	1	2	**3**	2	0	**2**	3	2	**5**	7	10	**17**
Total				1	2	**3**	**1.5**	1	3	**4**	3	0	**3**	3	4	**7**	9	10	**19**
Tremors	0	1	**1**				**0.5**										1	0	**1**

There was no increase in palpable masses observed.

Table of palpable masses in week 104 (Sponsor's)

	Controls 1	0.05 mg/kg	0.15 mg/kg	0.5 mg/kg	2.5 mg/kg	Controls 2
Males	3	2	2	0	2	2
Females	8	3	3	2	1*	5*

* until week 95

Body Weights

Body weights were recorded once during pre-treatment and then twice weekly for the first 12 weeks, then weekly until termination.

A statistically significant decrease in body weight was observed at doses greater than MLD with HD body weight decreasing from Week 3 by 4% and final decreases of 20% for males and 15% for females at sacrifice in Week 95. MHD female body weight was decreased by 17%.

Absolute body weight (g) and comparisons to control 1 (%) in week 103 (Sponsor's)

	Controls 1	0.05 mg/kg	0.15 mg/kg	0.5 mg/kg	2.5 mg/kg	Controls 2
Males	549	525 (95.6%)	492 (89.6%)	488 (88.9%)	439 (80%)	547
Females	333	312 (93.7%)	277 (83.2%)	278 (83.5%)	280* (84.1%)	330*

* in week 95

Food Consumption

Cage values determined once pre-treatment, weekly for the first 14 weeks, every 4 weeks until Week 78 and fortnightly thereafter.

A slight decrease in food consumption was observed in HD males from Week 1 with other male dose groups showing only trivial reduction. No difference between groups was noted in females.

Ophthalmoscopy

20 animals/sex/group (only 1 control) were examined pre-treatment with the same animals from control and HD groups during Week 53.

No treatment-related findings were observed.

Hematology

Samples were taken at study termination from all survivors. Additionally, blood and bone marrow smears were prepared from all early sacrifice animals and all survivors at scheduled necropsy. The following parameters were assessed.

List of blood parameters (Sponsor's)

erythrocytes	mean corpuscular hemoglobin	thrombocytes
hematocrit	mean corpuscular hemoglobin concentration	white blood cell count**
hemoglobin	mean corpuscular volume	red cell ditribution width
inclusion bodies*	reticulocytes	

* Counted if abnormalities were present.

** Total and differential.

MHD and HD males and MLD and MHD females had increased reticulocyte counts. The increases were dose-related, although it was not observed in HD females in which treatment was ceased from Week 75 on. The lack of an effect in HD females suggests some recovery during the following 20 weeks. Absolute reticulocyte count was 17% higher in Control 2 males compared to Control 1 males. Lymphocyte counts were significantly decreased in all treated groups.

Gross Pathology

A complete necropsy was conducted on all animals, including those animals found dead or sacrificed prematurely.

The Sponsor concluded that the observations of small thymus and seminal vesicles, enlarged spleen, foci in the brain and granular surface to the kidneys in HMD and HD animals were related indirectly to the "*generalized vasculopathy leading to poor general condition, increased severity of nephropathy, increased hematopoiesis in the spleen and hemorrhage in the brain.*"

At necropsy, blood filled body cavities were observed in males and females (see table below). The Sponsor attributed this finding in MHD and HD animals to vasculopathy and nephropathy, with no explanation for the lower dose group.

Gross Necropsy Findings in Rats Treated with FTY720 for Up to 104 Weeks

	MALE					FEMALE				
Dose (mg/kg/d)	**Controls***	**0.05**	**0.15**	**0.5**	**2.5#**	**Controls***	**0.05**	**0.15**	**0.5**	**2.5#**
Lung Not collapsed	1 (1+0)	0	0	1	4	0 (0+0)	1	0	1	3
Bronchi outflow	0 (0+0)	0	0	0	0	0 (0+0)	0	0	0	4
Enlarged spleen	5 (3+2)	2	1	3	6	1 (1+0)	2	5	4	5
Small thymus	1 (1+0)	0	1	0	4					
Granulated kidney	2 (0+2)	1	1	3	8	1 (0+1)	1	3	7	9
Cavity- blood/fluid	2 (1+1)	3	2	4	9	3 (2+1)	3	2	10	4
Brain-red foci	0 (0+0)	0	0	1	2	0 (0+0)	0	0	2	4
Small seminal vesicles	8 (5+3)	5	4	3	10	-	-	-	-	
Uterus- nodules/masses	-	-	-	-	-	4 (1+3)	4	7	6	4

*** Control groups 1 and 2 are combined; # Dosing stopped Weeks 75 and 99 for males and females, respectively.**

Histopathology

The tissues listed in the table below were taken from all animals. Immunohistochemistry (smooth muscle actin) was performed on some of the lung samples to determine the extent of the smooth muscle hypertrophy. Lung sections were examined using various stains (van Gieson, PAS, alcian blue) in order to further characterize the thickening seen in the pleura with H&E. Bone marrow smears were made, but not evaluated. "Wet tissues" were sent to a contract laboratory (b) (4) for processing.

Table of tissue for histological assessment (Sponsor's)

adrenals	liver	seminal vesicles
aorta	knee joint (femur/tibia)	skeletal muscle
bone marrow (in bone)	lacrimal glands	skin
brain	lungs	spinal cord
caecum	lymph node: axillary	spleen
clitoral gland	lymph node: mesenteric	sternum
colon	mammary area (with gland)	stomach
duodenum	nasal cavity (passage)	testes
epididymides	ovaries	thymus
esophagus	pancreas	thyroid with parathyroids
eyes with optic nerves	peripheral nerves (sciatic)	tongue
harderian glands	pituitary	trachea
heart	preputial gland	urinary bladder
ileum	prostate	uterus (with cervix)
jejunum	rectum	vagina
kidneys	salivary glands	zymbal glands

and all other organs/tissues showing macroscopic abnormalities (including palpable masses) during necropsy and animal identification.

Peer Review: Study pathologists were (b) (4). The study peer reviewer was (b) (4) Peer review was conducted on the draft report, histologic section review of all neoplasms and hyperplasias and all organs from all animals with neoplastic lesions as well as tissues from 10 animals/sex/group.

Observations of autolysis in males (Sponsor's)

SEX :						
DOSE GROUP:	01	02	03	04	05	06
NO.ANIMALS:	51	51	51	51	51	51
GENERAL OBSERVATIONS :	2	1	2	6	9	1
- Autolysis :	2	1	2	6	9	1

The Sponsor reports the incidence of autolysis as low in males (21) (see table above) and none in females. However, based on the individual line listings, the incidences are slightly higher 3, 3, 2, 7, 10 and 1 in males and 2, 1, 1, 2, 2 and 0 in females for control 1, LD MD, HD and control 2 groups, respectively. Although there are discrepancies, the numbers of affected animals are still low and, thus, did not prevent a definitive diagnosis.

Neoplastic: Rare tumors (squamous cell carcinoma of the preputial gland, malignant melanoma of the eye) were observed in individual males, but no individual had multiple tumors in any dose group (see table).

Table of some observed neoplasms including uncommon neoplasms

	MALE						FEMALE					
Neoplasms	**0**	**0**	**0.05**	**0.15**	**0.5**	**2.5**	**0**	**0.05**	**0.15**	**0.5**	**0**	**2.5**
Malignant lymphoma	1	1	2	1	4	1	2	1	1	1	0	1
Histiocytic sarcoma	1	1	0	0	1	0	0	0	1	0	0	0
Leukemia	0	0	0	0	0	1						
All Systemic	2	2	2	1	5	2	2	1	2	1	0	1
Hemangioma	0	1	0	0	1	0						
Hemangiosarcoma	0	2	0	0	1	1						
Hemangioma + hemangiosarcoma	0	3	0	0	2	1						
Preputial (clitoral) gland	(49)	(49)	(49)	(48)	(46)	(45)	(44)	(48)	(45)	(45)	(45)	(45)
carcinoma	0	0	1	0	1	1	0	0	0	0	0	0
Eye	51	51	51	51	51	(51)	(50)	(49)	(50)	(49)	(51)	(51)
malignant melanoma	0	0	0	0	0	1	0	0	0	0	0	1
Skin	51	51	51	51	51	(51)	(51)	(51)	(51)	(51)	(51)	(51)
sebaceous adenoma	0	0	0	0	0	1	0	0	0	0	0	0
fibrosarcoma	0	0	0	1	0	1	0	0	0	1	0	0
Bone	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
osteosarcoma	0	0	0	1	0	0	0	0	0	1	0	0
Thyroid gland	(51)	(51)	(51)	(51)	(51)	(50)	(50)	(50)	(50)	(50)	(51)	(49)
c-cell carcinoma	0	0	0	0	0	0	0	0	0	0	1	0
c-cell adenoma	1	2	11	6	2	2	3	1	2	5	3	1
Stomach	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(50)
squamous cell papilloma	0	0	0	0	0	0	0	0	0	1	0	0
Pituitary	(50)	(47)	(49)	(51)	(45)	(42)	(51)	(48)	(50)	(49)	(51)	(48)
carcinoma	0	0	0	0	0	0	0	1	0	1	0	0
adenoma	12	9	11	12	12	7	23	26	18	14	19	5

In females, there was a decreased incidence of pituitary adenomas in the treated animals compared to controls; this was suggested to be related to the decreased body weight gain. There were also proliferative findings in the uterus with a dose-dependent increase in incidence and severity of hyperplasia and neoplasia (even when considering that the HD females were stopped dosing at Week 75 and terminated at Week 95). The Sponsor did not consider these lesions to be treatment-related and concluded that

"*there is a negative trend towards less diffuse interstitial hyperplasia in the ovaries, mucinous hypertrophy of the vaginal epithelium and lower numbers of pituitary adenomas in the treated compared to the control animals, a perturbation in the hormonal status of these aged rats might be responsible for the increased incidence in groups 4 and 5*." The Sponsor suggests that the dose-dependent decrease in incidence of pituitary adenomas may be related to the uterine findings as these adenomas typically produce prolactin that would produce higher prolactin levels in controls and fewer estrogen-related lesions in the uterus, such as hyperplasia.

Significant Uterus lesions in female rats (n = 51) treated with FTY720 for up to 104 weeks

Dose (mg/kg/day)	Control 1	0.05	0.15	0.5	Control 2*	2.5#*
Polyps						
Stromal:	4	4	9	7	5	5
Glandular:	0	0	0	1	0	0
Hyperplasia						
Glandular:	10	7	7	13	10	12
Cystic endometrial:	0	0	1	2	0	0
Adenoma	1	0	1	1	0	0
Carcinoma	1	3	3	4	1	1
Adenoma+Carcinoma	2	3	4	5	1	1
Pooled hyperplasia and neoplasia	12	10	12	21	11	13

 # Treatment stopped Week 75, *Sacrificed early Week 95.

Non-Neoplastic: Histopathological findings in the lymphoid tissues were expected due to the pharmacologic effect of FTY720. The observations described included atrophy of the spleen, lymph nodes, and thymus. A dose-dependent vasculopathy was observed in all treated animals and described as hypertrophy/hyperplasia of the middle and outer layers of blood vessels, fibrinoid necrosis and inflammation with occasional thrombi affecting most organs. The organs most affected were testes, pancreas, heart, mesentery, tongue, stomach, small and large intestine, spleen, kidney, brain and uterus. The Sponsor stated that the lesion, although statistically significant at the LD in females when compared to Control 1, was not biologically significant when compared to Control 2. While the pharmacologic activity of FTY720 may result in vasculopathy and a slight degree is observed in long chronic/carcinogenicity studies due to normal aging in rats, the level, severity and organ sites observed in the present study appear greater and more extensive than expected and is, therefore, most likely treatment-related. Similar to other toxicology studies, slight smooth muscle hypertrophy at the broncho-alveolar junctions was observed in MHD and HD males and all female treatment groups. Dose-dependent bile duct hyperplasia was observed in all treated animals that increased in incidence and severity. In the brain there was a dose-dependent increased incidence and severity of focal/multifocal mineralization in all treatment groups. The Sponsor states that the observations were not dose-dependent in females; however, treatment was stopped early in HD females.

Non-Neoplastic Lesions in Rats Treated Orally with FTY720 for Up to 104 Weeks

	MALE							FEMALE					
Group (mg/kg/d)	**0**	**0**	**Ave**	**0.05**	**0.15**	**0.5**	**2.5[@]**	**0**	**0.05**	**0.15**	**0.5**	**0***	**2.5[#]***
Spleen	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Increased hematopoiesis:	11	19	**15**	17	25	24	30	3	4	12	17	2	15
Lymphoid atrophy:	3	7	**5**	13	23	21	9	2	33	40	39	4	30
Thymus	(43)	(47)		(48)	(50)	(47)	(49)	(49)	(49)	(48)	(49)	(49)	(46)
Tinctorial change:	0	0	**0**	39	42	37	27	0	41	39	43	0	25
Enlarged medulla:	0	0	**0**	5	8	1	3	0	3	0	1	1	3
Atrophy:	6	6	**6**	5	6	9	12	5	12	8	18	11	17
Lymphocytolysis:	2	0	**1**	0	1	0	5	0	0	0	0	0	0
Mesenteric Lymph Node	(51)	(51)		(51)	(51)	(51)	(50)	(51)	(51)	(50)	(50)	(50)	(48)
Lymphoid atrophy:	3	8	**5.5**	42	49	39	28	5	39	43	40	10	25
Sinusoidal depletion:	0	0	**0**	46	49	45	29	2	35	36	41	0	27
Sinus dilatation:	5	4	**4.5**	18	20	19	12	1	6	8	7	2	2
Histiocytosis:	0	0	**0**	0	0	0	0	2	11	2	7	1	8
Axillary Lymph Node	(44)	(46)		(47)	(49)	(50)	(44)	(48)	(50)	(47)	(47)	(45)	(38)
Lymphoid atrophy:	0	0	**0**	8	12	20	30	10	14	20	26	2	15
Sinusoidal depletion:	0	0	**0**	20	18	20	2	0	0	0	0	0	0
Sinus dilatation:	2	0	**1**	7	11	16	15	0	5	5	6	0	2
Lungs	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Sm. muscle hypertrophy:	0	1	**0.5**	0	0	11	38	0	2	5	6	0	22
Pleural thickening:	1	0	**0.5**	1	9	26	32	3	0	2	5	0	(8)
Osseous metaplasia:	1	2	**1.5**	2	1	6	8	0	0	0	0	0	0
Kidney	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Nephropathy:	21	29	**25**	16	16	26	43	6	12	22	31	2	35
Liver	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Hematopoiesis:	1	1	**1**	0	0	2	3	0	2	5	4	1	6
Bile duct hyperplasia:	4	0	**2**	17	21	18	15	16	39	38	38	17	22
Heart	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(50)	(51)	(51)	(51)	(51)
Degeneration/Fibrosis:	32	25	**28.5**	31	22	30	44	13	14	16	28	8	26
Blood vessels	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Vasculopathy:	1	7	**4**	4	5	22	43	0	6	22	29	3	33
Brain	(51)	(51)		(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)	(51)
Mineralization:	1	0	**0.5**	9	13	21	27	0	0	1	14	0	5

 # Treatment stopped Week 75, [@]Dosing stopped Week 99, *Sacrificed early Week 95.

Toxicokinetics

Blood samples were taken during Weeks 4, 26 and 52 from 1 animal/sex/group/time point at 1, 4, 7 and 24 hours post-dosing. Samples were also taken during Week 101 but were not analyzed.

The Sponsor stated that drug was not detected in the control animals. Plasma exposure increased over time in all dose groups. The toxicokinetics appear to be linear for each sampling period, although there was accumulation between Week 4 and 26 that appeared to plateau by Week 52. T_{max} was between 4-7 hours and plasma exposure was slightly increased in females compared to males.

Sponsor's table of toxicokinetic parameters for FTY720 in males:

Dose	Sampling period	t_{max}	C_{max}	C_{max}/dose	AUC(0-24h)	AUC(0-24h)/dose
0.05 mg/kg/day	Week 4	7	1.785	35.7	32.4	649
	Week 26	4	3.491	69.8	68.5	1370
	Week 52	7	3.158	63.2	66.8	1337
0.15 mg/kg/day	Week 4	7	5.819	38.8	105	701
	Week 26	4	10.263	68.4	195	1300
	Week 52	7	10.481	69.9	193	1289
0.5 mg/kg/day	Week 4	7	23.042	46.1	447	894
	Week 26	4	29.857	59.7	577	1155
	Week 52	7	32.138	64.3	665	1330
2.5 mg/kg/day	Week 4	7	119.391	47.8	2233	893
	Week 26	7	162.637	65.1	3249	1299
	Week 52	7	167.354	66.9	3149	1260

t_{max} is expressed in h. C_{max} is expressed in ng/mL. AUC(0-24h) is expressed in ng·h/mL. AUC(0-24h)/dose is expressed in (ng·h/mL)/(mg/kg/day). For calculation of AUC(0-24h), the concentration at 0 was replaced by the concentration measured at 24h.

Sponsor's table of toxicokinetic parameters for FTY720 in females:

Dose	Sampling period	t_{max}	C_{max}	C_{max}/dose	AUC(0-24h)	AUC(0-24h)/dose
0.05 mg/kg/day	Week 4	7	2.356	47.1	42.1	841
	Week 26	7	3.537	70.7	67.4	1348
	Week 52	4	3.644	72.9	71.6	1432
0.15 mg/kg/day	Week 4	7	6.253	41.7	111	741
	Week 26	4	10.655	71.0	209	1395
	Week 52	7	10.554	70.4	213	1418
0.5 mg/kg/day	Week 4	7	26.733	53.5	494	988
	Week 26	4	35.218	70.4	591	1183
	Week 52	7	44.815	89.6	841	1683
2.5 mg/kg/day	Week 4	7	124.765	49.9	2473	989
	Week 26	7	166.942	66.8	3308	1323
	Week 52	7	198.317	79.3	3748	1499

t_{max} is expressed in h. C_{max} is expressed in ng/mL. AUC(0-24h) is expressed in ng·h/mL. AUC(0-24h)/dose is expressed in (ng·h/mL)/(mg/kg/day). For calculation of AUC(0-24h), the concentration at 0 was replaced by the concentration measured at 24h.

Second Peer Review:
A second full peer review was requested by the Agency. The review was performed by an external pathologist between 20 Feb 2006 and 24 Jan 2007: (b) (4) The peer review included a review of the histological slides from all tumors and pre-neoplastic findings (focal hyperplasias), slides from: thymus, spleen, axillary and mesenteric lymph nodes, lung, kidneys, liver, pancreas, heart, brain and all tissues from 191 animals.

Neoplastic Tumors were found in the thyroid gland, c-cell adenoma, in LD and MLD males; however, the trend was not significant (p-value of 0.6901). Changes observed for males from the initial study report include hemangiomas and hemangiosarcomas (see table) and the deletion of adrenal cortical carcinoma in males. There appears to be inconsistencies in hemangioma/hemangiosarcoma data. In the non-neoplastic lesion table for males (pages 13-14) mesenteric lymph node hemangioma incidences changed, but the number of hemangiosarcoma remained the same, however when reading the line listings (pages 29-32), animals 356, 455 and 537 have a diagnosis change from hemangiosarcoma to hemangioma.

Table of changes observed in Hemangiomas and hemangiosarcomas

			MALE			
Neoplasms	**0**	**0**	**0.05**	**0.15**	**0.5**	**2.5**
Hemangioma	0	(1) **2**	0	0	(1) **2**	(0) **1**
Hemangiosarcoma	0	2	0	0	1	1
Hemangioma + hemangiosarcoma	0	(3) **4**	0	0	(2) **3**	(1) **2**

Old incidences in parenthesis, changed values after peer review in bold.

Changes in proliferative findings in the uterus changed little with a carcinoma reverting to an adenoma in the LD group and adenoma reverting to hyperplasia (grade 4) in the MLD group

Significant Uterus lesions in female rates treated with FTY720 for up to 104 weeks

Dose (mg/kg/day)	**Control 1**	**0.05**	**0.15**	**0.5**	**Control 2***	**2.5#***
Polyps						
Stromal:	4	4	9	7	5	5
Glandular:	0	0	0	1	0	0
Hyperplasia						
Glandular:	10	7	(7) **8**	13	10	12
Cystic endometrial:	0	0	1	2	0	0
Adenoma	1	(0) **1**	(1) **0**	1	0	0
Carcinoma	1	(3) **2**	3	4	1	1
Adenoma+Carcinoma	2	3	4	5	1	1
Pooled hyperplasia and neoplasia	12	10	12	21	11	13

 # Treatment stopped Week 75, *Sacrificed early Week 95. Old incidences in parenthesis, changed values after peer review in bold.

Non-neoplastic: Small changes were also observed in some non-neoplastic histopathological findings, but these were minor (see table below).

Non-Neoplastic Lesions in Rats Treated Orally with FTY720 for Up to 104 Weeks

	MALES (n = 51)							FEMALES (n = 46-49)					
Group (mg/kg/d)	**0**	**0**	**Ave**	**0.05**	**0.15**	**0.5**	**2.5@**	**0**	**0.05**	**0.15**	**0.5**	**0***	**2.5#***
Mesenteric LN													
Lymphoid atrophy:	3	8	5.5	42	49	39	28	5	(39) **40**	43	40	10	25
Sinusoidal depletion:	0	0	0	46	49	45	29	2	35	36	41	0	27
Sinus dilatation:	5	4	4.5	18	20	19	12	1	6	8	(7) **8**	(2) **3**	(2) **4**
Histiocytosis:	0	0	0	0	0	0	0	2	11	2	7	1	8
Lungs													
S. muscle hypertrophy:	0	1	0.5	0	0	11	38	0	2	5	6	0	(22) **23**
Pleural thickening:	1	0	0.5	1	9	26	32	3	0	2	5	0	8
Osseous metaplasia:	1	2	1.5	2	1	6	8	0	0	0	0	0	0
Liver													
Hematopoiesis:	1	1	1	0	0	2	3	0	2	5	4	1	6
Bile duct hyperplasia:	4	0	2	17	(21) **18**	(18) **17**	15	16	39	38	(38) **37**	(17) **16**	(22) **19**
Heart													
Degeneration/Fibrosis:	(32) **33**	25	29	(31) **33**	(22) **23**	(30) **31**	44	13	14	16	28	(8) **9**	26

 # Treatment stopped Week 75, @Treatment stopped Week 99, *Sacrificed early Week 95. Old incidences in parenthesis, changed values after peer review in bold.

After the peer review, the Sponsor maintained the conclusion that "*Oral administration of FTY720 to rats for 104 weeks showed no tumorigenic potential up to the highest dose level of 2.5 mg/kg/day*". This does not appear to be a reasonable conclusion on several levels. First, only rats up to 0.5 mg/kg/day were administered FTY720 for the full 104 week treatment period, as both HD males and female treatment was stopped and HD females were sacrificed before Week 104. Second, the uterine lesions are probably treatment related, as the incidence and severity of hyperplasia and neoplasia was greater in MHD and the HD females stopped treatment after Week 75.

Overall summary

Treatment of male and female animals at 2.5 mg/kg experienced high levels of mortality with 29.4% and 62.8% dead in the first 78 weeks with treatment stopped at Weeks 99 and 75, respectively. This suggests that treatment at 2.5 mg/kg exceeded an MTD, as there was also an increase in mortality for HMD animals. Although only slight for males, recumbency and breathing difficulties were observed and body weights decreased at 0.5 mg/kg.

There was an increase in reticulocytes that was not due to anemia, as red blood cell parameters were unaffected and was most likely related to increased splenic hematopoiesis and vasculopathy of blood vessels. Lymphocyte counts were significantly decreased in all treated animal groups as would be expected from the pharmacological action of FTY720 and resulted in atrophy of the lymphatic organs. After a second peer-review (requested by the Agency), only minor changes occurred in the histopathological observations. Although autolysis of organs appeared to be under reported, the level of autolysis did not prevent diagnosis. There was vasculopathy that due to the extensive nature through various organs is most likely treatment related. Bile

duct hyperplasia was observed in all treated animals that increased in incidence and severity and a dose-dependent increase in incidence and severity of focal/multifocal mineralization in the brain of all treatment groups. Also, as previously reported in other toxicology studies, there was smooth muscle hypertrophy at the broncho-alveolar junctions from 0.5 mg/kg in males and 0.05 mg/kg in females.

Few neoplastic changes were reported, suggesting that FTY720 was not tumorigenic. The Sponsor concluded that "*oral administration of FTY720 to rats for 104 weeks showed no tumorigenic potential up to the highest dose level of 2.5 mg/kg/day, representing a maximal exposure (AUC_{0-24h}) of 2230 to 3750 ng·h/ml*", the highest dose that was treated for the full 104 week period was 0.5 mg/kg, associated with a plasma AUC of 665-841 ng*h/ml, and 10 times the proposed human dose of 0.5 mg on a mg/m^2 basis. The incidences of neoplastic uterine findings were dose-dependent in incidence and severity, with an increase in hyperplasia observed at 0.5 mg/kg that suggests a treatment-related effect. However, the effect was not statistically significant.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: An oral combined fertility and early embryonic development study in rats treated with Y-32720

Study no.:	PCS-r7027
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 September 1994
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Y-32720, Lot № N, 100.1% purity

Key Study Findings

- There were deaths of dams in all dose groups.
- Duration of mating, copulation and fertility indices; corpora lutea, implantations, and viable fetuses numbers were unaffected by FTY720 treatment.

Methods

Doses:	0, 1, 3 and 10 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	4 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	Distilled water for injection
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	20
Age:	Males - 6 weeks Females - 7 weeks
Weight:	Males - 193.4-207.4 g Females - 164.9-181.6 g
Study design:	M were dosed for 28 days prior to mating and then until the day prior to sacrifice, Day 5 or 6 after successful copulation or at the end of the mating period if copulation was not successful. F were dosed for 15 days prior to mating, during mating period and until Day 7 of gestation (if copulation was successful) or until the day prior to the last day of mating (if copulation was unsuccessful)

Observations and results

Mortality

One M at the LD was found dead on Day 23. Four F at the MD were found dead on Days 5, 13, 15 and gestation Day 1; no clinical signs were observed in these F. One F at the HD was found dead on Day 13; transient salivation was observed in this F. Bubbly fluid was found in the trachea and larynx of all animals so the Sponsor concluded that the deaths were due to dosing errors.

Clinical Signs

In M, signs were observed daily from Day 0 of treatment to sacrifice. In F, signs were observed daily prior to treatment, during pre-mating period to the day of caesarean section or necropsy.

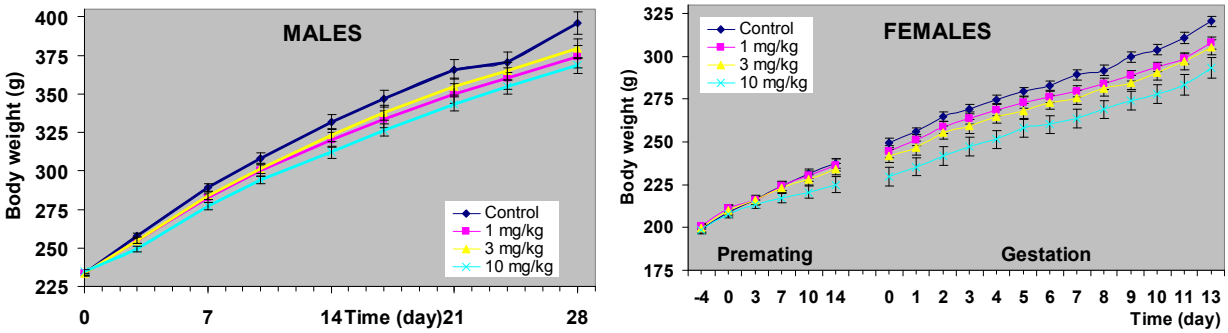
No clinical signs were recorded for animals at the LD and MD. Only occasional (0-5 observation/day) salivation was observed in animals at the HD. One F showed emaciation from Day 11 in the premating period.

Body Weight

In M, body weights were recorded twice a week during the premating period. In F, body weights were recorded 4 days pre-treatment and twice a week during the premating period. During gestation, body weights were recorded daily from Day 0-11 and on Day 13.

In M, body weight gain was significantly decreased at the HD (17.7% on Day 28). In F, body weights were reduced at the HD (~5%). During gestation Days 9-13 body weight gain was reduced in all treated groups.

Figure of body weights



Food Consumption

Food consumption was recorded weekly during the premating period.

Total food consumption was decreased in all treated M (5.6%, 6.0% and 7.7% at LD, MD and HD, respectively). In F, food consumption was reduced on Day 7 and Day 10 at the MD and HD. During gestation, food consumption was reduced in F on Day 9 at the LD, on Days 7 and 9 at the MD and on Days 2-5, 7, 9 and 11 at the HD.

Gross findings

On gestation Day 13, F were sacrificed and visceral organs examined macroscopically.

In M, atrophy of spleens occurred in a dose-dependent manner with 15, 17 and 18 incidences at the LD, MD and HD, respectively. There was testes softening and atrophy seen in 1 M from each dose group and atrophy of the epididymis of 2 M at the LD and 1 M at the HD and green mucous abscess observed in 1 M at the MD. Two M at the HD had atrophy of the prostate and seminal vesicle. Atrophy of the spleen was observed in F, with 3 incidences at the MD and HD.

Histopathology

Testes and epididymis were fixed in Bouin's solution. Other organs taken and fixed in 10% neutral buffered formalin for M were spleen, prostate, seminal vesicles and macroscopic findings. After fertility parameters had been recorded in F, uteri, ovary, embryos, kidneys and spleen were fixed in 10% neutral buffered formalin. Organs taken from control animals were thymus, heart, lungs, liver, kidneys, adrenals, spleen, urinary bladder, prostate and seminal vesicles.

Apart from fertility parameters there were no histopathological data submitted.

Fertility Parameters

Sperm was determined on the caudal epididymis. Uterus and ovary were removed at necropsy and the number of corpora lutea, implantations, viable embryos and dead embryos recorded.

In M, there was no change in sperm counts, although 1 M at the LD and 1 M at the HD had low (2.3, ~1.4% of mean) counts. Duration of mating, copulation and fertility indices were unaffected. Numbers of corpora lutea, implantations, and viable fetuses were similar between groups. However, pre-implantation losses were elevated by 128% (38 in 19 dams; 2/dam) in F at the LD and 59% (21 in 16 dams; 1.3/dam) in F at the HD, compared to control F (16 in 18 dams 0.89/dam). Only 14 dams at the MD were evaluated; below the ICH-recommended 16-20.

9.2 Embryonic Fetal Development

Study title: An oral embryo-fetal development study in rats treated with Y-32720

Study no:	PCS-r7763
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5 June 1996
GLP compliance:	Yes ((b) (4), 1997)
QA statement:	Yes (Dated: 25 June 1997; Amended 26 Aug 1998)
Drug, lot #, and % purity:	Y-32720, Lot № O, 100.1% purity

Key Study Findings

- The NOAEL for the dams in this study was 1 mg/kg.
- There were no viable fetuses at 10 mg/kg/day.
- Fetal heart-vessel and skeletal findings were observed at 1 and 3 mg/kg/day.
- The NOAEL for fetuses in this study was less than 1 mg/kg.

Methods

Doses:	0, 1, 3 and 10 mg/kg
Frequency of dosing:	Daily
Dose volume:	4 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	20 pregnant females/group
Age:	11-12 weeks
Weight:	Females - 230.9-297.7 g at gestation Day 0
Study design:	Y-32720 was administered daily on gestation Days 7-17

Observations and results

Mortality

No unscheduled deaths occurred in dams.

Clinical Signs
Observations were performed once on Day 0 and daily during gestation Days 6-20.

No clinical signs were recorded for animals at the LD and MD. Salivation was observed in F at the HD on gestation Days 7-10, 12-14 and 16-18.

Body Weight
Body weight recordings were taken once on Day 0 and daily during gestation Days 6-20.

Decrease in body weight was observed in F at the MD during gestation Days 18-20 (7.9-14.9%) and in F at the HD during gestation Days 17-20 (5.5-16.8%).

Food Consumption
Food consumption recordings were taken once on Day 0 and daily during gestation Days 6-20.

Decrease in food consumption occurred in F at the LD on gestation Day 18, at MD during Days 10, 12, and 14-20 and at the HD during Days 8-12, 14 and 16-18.

Necropsy
On gestation Day 20, F were sacrificed and visceral organs examined macroscopically. The uterus was removed for assessment of fertility parameters. The spleen was removed and weighed in all groups. The thymus, heart, lungs, liver, kidneys, adrenals, spleen and reproductive organs were removed from 6 animals from the control group and fixed in 10% neutral buffered formalin.

Atrophy of the spleen occurred in F at the MD and HD and spleen weight was decreased in all treated F. A red brown fluid was observed in the uterus of 2 F at the MD, with uterine hemorrhage occurring in F at the MD (8 dams) and HD (11 dams).

Cesarean Section Data
Embryos and fetuses were classified into implantation sites, resorptions, placental remnants, macerated fetuses, dead fetuses and viable fetuses.

There were no differences in numbers of corpora lutea, implantations and pre-implantation losses. However, post-implantation loss was increased in dams at the LD and MD and there was total post-implantation loss in all dams at the HD that were mainly early resorptions. On average, dams at the MD had less than 1 fetus/litter and fetal weights at the MD and placental weights at the LD and MD were decreased.

Offspring (Malformations, Variations, etc.)
Viable fetuses were weighed and approximately one-half of each litter fixed in ethyl alcohol and stained with alizarin red S (Dawson's method). The other half were preserved in 10% neutral buffered formalin and fixed in Bouin's solution.

External observations: Edema was present in 3/243 fetuses at the LD and 2/17 fetuses at the MD. The body weight of fetuses at the MD and placental weight at the LD and MD were decreased.

Visceral findings: There was an increase in total malformations in fetuses at the LD and MD. These included at the LD and MD; increased persistent truncus arteriosus, ventricular septal defect and left umbilical artery increased and at the LD incidence of thymic remnant in the neck and supernumerary coronary orifice. Abnormal bifurcation of the left subclavian artery was increased at the MD.

Table of visceral observations (Sponsor's)

Group and dose	Y-32720 0mg/kg	Y-32720 1mg/kg	Y-32720 3mg/kg
No. of fetuses examined	140	117	6
No. of fetuses with anomalies	16(11.43)	69(58.97)**	4(66.67)**
Thymic remnant in the neck	12(8.57)	47(40.17)**	0
Right aortic arch	0	1(0.85)	0
Abnormal bifurcation of LSA	0	1(0.85)	1(16.67)*
Persistent truncus arteriosus	0	18(15.38)**	3(50.00)**
Supernumerary coronary orifice	0	5(4.27)*	0
Ventricular septal defect	0	18(15.38)**	4(66.67)**
Dilatation of renal pelvis	3(2.14)	6(5.13)	0
Left umbilical artery	1(0.71)	8(6.84)*	2(33.33)**
Bilateral umbilical artery	0	2(1.71)	0
No. of fetuses with variations	0	0	0

Values in parentheses represent percentages to the number of fetuses examined.
LSA:Left subclavian artery
*:P<0.05,**:P<0.01(significant difference from control)

Skeletal findings: Significantly delayed ossification of the pubis was increased in fetuses at the LD and MD and an increase in 13th rib shortening occurred at the LD. The numbers of sternebra, sacral and caudal vertebrae, and metacarpus at the LD and MD were significantly decreased and the number of metatarsals at the MD was decreased.

Table of skeletal observations (Sponsor's)

Group and dose	Y-32720 0mg/kg	Y-32720 1mg/kg	Y-32720 3mg/kg
No. of fetuses examined	152	126	11
No. of skeletal anomalies	1(0.66)	2(1.59)	0
Absence of TVA	0	1(0.79)	0
Absence of TVB	0	1(0.79)	0
Wavy rib	1(0.66)	1(0.79)	0
Absence of the 13th ribs	0	1(0.79)	0
Curvature of radius	0	1(0.79)	0
No. of skeletal variations	61(40.13)	59(46.83)	9(81.82)**
Retarded ossification of CVA	5(3.29)	7(5.56)	3(27.27)
Bilateral twin thoracic bodies	3(1.97)	0	0
Lumbar rib	4(2.63)	0	0
Shortening of the 13th rib	3(1.97)	11(8.73)*	0
Retarded ossification of OB	27(17.76)	21(16.67)	1(9.09)
Retarded ossification of SVA	45(29.61)	39(30.95)	6(54.55)
Retarded ossification of ischia	2(1.32)	8(6.35)	1(9.09)
Retarded ossification of pubis	13(8.55)	49(38.89)**	7(63.64)**
Retarded ossification of ZB	3(1.97)	0	0

Values in parentheses represent percentages to the number of fetuses examined.
TVA:Thoracic vertebral arches
TVB:Thoracic vertebral bodies
CVA:Cervical vertebral arches
OB:Occipital bones
SVA:Sacral vertebral arches
ZB:Zygomatic bone
*:P<0.05, **:P<0.01(significant difference from control)

Study title: An additional oral embryo-fetal development study in rats treated with Y-32720

Study no: PCS-r7797
Study report location: 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: 14 October 1996
GLP compliance: Yes (1997)
QA statement: Yes (Dated: 23 Jul 1997; Amended 18 Aug 1998)
Drug, lot #, and % purity: Y-32720, Lot № O, 100.1% purity

Key Study Findings

- The total incidence of visceral malformations of the thymus and cardiovascular system in fetuses was significantly increased dose-dependently at 0.1 and 0.3 mg/kg/day. There was also significant increase in the incidence of left umbilical artery in fetuses at 0.03 mg/kg/day.
- Fetuses at 0.3 mg/kg/day had a significant increase in the incidence of bilateral twin thoracic bodies. Fetuses at 0.03 and 0.1 mg/kg/day were not analyzed for skeletal findings.

- The NOAEL for fetuses in this study was 0.03 mg/kg, although, fetuses 0.03 and 0.1 mg/kg/day were not analyzed for skeletal findings.

Methods

Doses:	0, 0.03, 0.1 and 0.3 mg/kg
Frequency of dosing:	Daily
Dose volume:	4 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	20 pregnant females/group
Age:	11-12 weeks
Weight:	Females - 225.5-281.9 g at gestation Day 0
Study design:	Y-32720 was administered daily on gestation Days 7-17

Observations and Results for Dams

Mortality

No dam deaths occurred.

Clinical Signs

Observations were performed once on Day 0 and daily during gestation Days 6-20.

No clinical signs were recorded for any treated animal.

Body Weight

Body weight recordings were taken once on Day 0 and daily during gestation Days 6-20.

There were no body weight changes observed.

Food Consumption

Food consumption recordings were taken once on Day 0 and daily during gestation Days 6-20.

There were no food consumption changes observed.

Necropsy

On gestation Day 20, F were sacrificed and visceral organs examined macroscopically. The uterus was removed for assessment of fertility parameters, the spleen removed and weighed and the thymus, heart, lungs, liver, kidneys, adrenals, spleen, uterus and ovary removed from 6 control females and fixed in 10% neutral buffered formalin.

No macroscopic findings were observed in any treated animal. In F, spleen weight at the MD and HD was decreased.

Cesarean Section Data
Embryos and fetuses were classified into implantation sites, resorptions, placental remnants, macerated fetuses, dead fetuses and viable fetuses.

There were no differences in numbers of corpora lutea, implantations, pre-implantation losses, post-implantation losses and viable fetuses. There was an increase in fetal weight at the HD, but no change at the LD and MD or in placental weight.

Offspring (Malformations, Variations, etc.)
Viable fetuses and placentas were weighed and approximately one-half of each litter fixed in ethyl alcohol and stained with alizarin red S (Dawson's method). The other half were soaked in 10% neutral buffered formalin and fixed in Bouin's solution. No skeletal examination was performed on fetuses from dams at the LD and MD.

External observations: No findings in fetuses were observed at any dose.

Visceral findings: Total incidence of visceral malformations was significantly increased in fetuses at the MD and HD. There was incidence of thymic remnant in the neck at the HD, persistent truncus arteriosus, ventricular septal defect and dilation of the renal pelvis at the MD and HD. The observation of the left umbilical artery was significantly at the LD, but was not considered related to FTY720, it was not observed at the MD and HD. There were incidences of right aortic arch and persistent right azygos vein, but was not significant compared to control fetuses.

Table of visceral observations (Sponsor's)

Group and dose	Y-32720 0mg/kg	Y-32720 0.03mg/kg	Y-32720 0.1mg/kg	Y-32720 0.3mg/kg
No. of fetuses examined	146	141	139	136
No. of fetuses with anomalies	13(8.90)	14(9.93)	31(22.30)*	62(45.59)**
Thymic remnant in the neck	11(7.53)	12(8.51)	26(18.71)	51(37.50)**
Right aortic arch	0	0	0	2(1.47)
Retroesophageal RSA	1(0.68)	0	0	0
Persistent truncus arteriosus	0	0	1(0.72)	6(4.41)*
Supernumerary coronary orifice	1(0.68)	0	0	0
Ventricular septal defect	0	0	2(1.44)	15(11.03)**
Persistent right azygos vein	0	0	0	1(0.74)
Dilatation of renal pelvis	0	0	1(0.72)	5(3.68)*
Left umbilical artery	0	4(2.84)*	3(2.16)	2(1.47)

Values in parentheses represent percentages to the number of fetuses examined.
RSA: right subclavian artery
*: $p<0.05$, **: $p<0.01$ (significant difference from control)

Skeletal findings: Skeletal variations observed in fetuses at the control and HD (there were different numbers of fetuses examined) included retarded ossification of the zygomatic bone, cervical and sacral vertebral arches, thoracic bodies, Ischia and pubis and a shortening of the 13th rib. Fetuses, at the HD, had a significant increase in the incidence of bilateral twin thoracic bodies and decrease in the incidence of retarded ossification of occipital bone. Fetuses at the LD and MD were not analyzed.

Table of skeletal observations (Table on page 28, PCS-r7797 is incorrect)

Group and dose (mg/kg)	0		0.03	0.1	0.3	
№ of dams with fetuses	20		20	20	20	20
№ of fetuses examined	152	100%	None	None	147	100%
№ of skeletal anomalies	0 (0)		NE	NE	0 (0)	
№ of skeletal variations	38 (13)		NE	NE	50 (17)	
Retarded ossification of CVA	13 (7)	8.6%	NE	NE	15 (7)	10.2%
Bilateral twin thoracic bodies	0 (0)	0.0%	NE	NE	8 (5)	5.4%
Retarded ossification of TB	3 (2)	2.0%	NE	NE	11 (7)	7.5%
Shortening of the 13th rib	2(2)	1.3%	NE	NE	6 (4)	4.1%
Retarded ossification of OB	13 (8)	8.6%	NE	NE	1 (1)	0.7%
Retarded ossification of SVA	28 (13)	18.4%	NE	NE	12 (10)	8.2%
Retarded ossification of ischia	6 (3)	3.9%	NE	NE	3 (2)	2.0%
Retarded ossification of pubis	16 (8)	10.5%	NE	NE	19 (11)	12.9%
Retarded ossification of ZB	3 (2)	2.0%	NE	NE	1 (1)	0.7%

NE – not examined, parenthesis - № of litters.

Study title: An oral embryo-fetal development dose-range finding study in rabbits treated with Y-32720

Study no: PCS-r7873
Study report location: 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: 15 February 1997
GLP compliance: Yes (1997)
QA statement: Yes (Dated: 3 Oct 1997; Amended 26 Aug 1998)
Drug, lot #, and % purity: Y-32720, Lot № S, 100.0% purity

Key Study Findings

- The NOAEL for the dams in this study was greater than 3 mg/kg.
- The NOAEL for fetuses in this study could not be determined as not all fetuses were examined. Fetuses at the 0.01 and 0.03 mg/kg/day were not examined for visceral findings and fetuses at the 0.01, 0.03 and 0.3 mg/kg/days were not examined for skeletal findings.

Methods

Doses:	0, 0.01, 0.03, 0.3 and 3 mg/kg
Frequency of dosing:	Daily
Dose volume:	4 ml/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Distilled water
Species/Strain:	Rabbit/Kbl:JW
Number/Sex/Group:	7 pregnant females/group
Age:	Females: 17-18 weeks Males: 19-20 weeks
Weight:	Females: 2.86-3.60 kg at gestation Day 0
Study design:	Y-32720 was administered daily on gestation Days 6-18

Observations and Results for Dams

Mortality

One dam at the MLD died on Day 16 after a decrease in body weight on Day 15 and decreased fecal and urine output on Day 16. A dosing error was suggested because of lung findings that were not fully explained.

Clinical Signs

Observations were performed once on Day 0 and daily during gestation Days 5-28.

No clinical signs data were presented

Body Weight

Body weight recordings were taken once on Day 0, daily during gestation Days 6-19 and on Days 22, 25 and 28.

There was no body weight change observed.

Food Consumption

Food consumption recordings were taken once on Day 0 and daily during gestation Days 6-19 and on Days 22, 25 and 28.

There was no food consumption change observed.

Necropsy

On gestation Day 28, dams were sacrificed and visceral organs examined macroscopically. The ovary and uterus were removed for assessment of fertility parameters; the heart, liver, lungs, kidneys, spleen, adrenals and ovary were removed and weighed and the heart, lungs, liver, kidneys, adrenals, spleen, uterus and ovary of all control females were fixed in 10% neutral buffered formalin. However, all organs fixed were discarded before histological examination.

In dams, adrenal weight was decreased at the MHD and spleen weight decreased at the HD.

Cesarean Section Data
Embryos and fetuses were classified into implantation sites, resorptions, placental remnants, macerated fetuses, dead fetuses and viable fetuses, when the implantation site was unclear; Sumida's method was used to count the number of implantations.

One control dam spontaneously aborted all fetuses on Day 21. In treated dams, no differences in numbers of corpora lutea, implantations and pre-and post-implantation losses, number of live fetuses, and fetal and placental weight were apparent. There was a non-statistically significant dose-dependent increase in resorptions with 2, 3, 3, 6 and 9 resorptions for control, LD, MLD, MHD and HD, respectively.

Offspring Observations
Fetuses and placenta were weighed and approximately one half of the fetuses fixed in ethyl alcohol and stained with alizarin red S (Kawamura's method). The other half were preserved in 10% neutral buffered formalin and fixed in Bouin's solution (Nishimura's method). No visceral examination was performed on fetuses at the LD and MLD and no skeletal examination was performed on fetuses at the LD, MLD and MHD.

External observations: There were no external findings.

Visceral findings: There was an increase in incidence of visceral malformations in fetuses at the HD, these included thymic remnant in the neck and in 1 fetus persistent truncus arteriosus, ventricular septal defect, abnormal bifurcation of the left carotid artery, bifid apex of the heart and absence of the left umbilical artery was observed.

Table of visceral observations (Sponsor's)

Group and dose	Y-32720 0mg/kg	Y-32720 0.3mg/kg	Y-32720 3mg/kg
No. of fetuses examined	23	24	29
No. of fetuses with anomalies	4(17.39)	8(33.33)	14(48.28)
Thymic remnant in the neck	1(4.35)	4(16.67)	10(34.48)*
Bifid apex of heart	0	0	1(3.45)
Abnormal bifurcation of LCCA	0	0	1(3.45)
Persistent truncus arteriosus	0	0	1(3.45)
Supernumerary coronary orifice	3(13.04)	4(16.67)	3(10.34)
Ventricular septal defect	0	0	1(3.45)
Absence of left umbilical artery	0	0	1(3.45)

Values in parentheses represent percentages to the number of fetuses examined.
LCCA : left comman carotid artery
* : p<0.05(significant difference from control)

Skeletal findings: No significant skeletal findings were observed in fetuses at the HD.

Table of skeletal observations (Sponsor's)

Group and dose	Y-32720 0mg/kg	Y-32720 3mg/kg
No. of fetuses examined	25	32
No. of skeletal anomalies	0	1(3.13)
Fusion of sternebrae	0	1(3.13)
No. of skeletal variations	7(28.00)	13(40.63)
Cervical ribs	0	1(3.13)
Retarded ossification of OH	2(8.00)	1(3.13)
Lumbar rib	6(24.00)	11(34.38)
Accessory sternebrae	0	2(6.25)

Values in parentheses represent percentages to the number of fetuses examined.
OH : os hyoideum

Study title: An additional oral embryo-fetal development dose-range finding study in rabbits treated with Y-32720

Study no: PCS-r7912
Study report location: 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: 15 May 1997
GLP compliance: Yes (1997)
QA statement: Yes (Dated: 10 Dec 1997; Amended 21 Aug 1998)
Drug, lot #, and % purity: Y-32720, Lot № S, 100.0% purity

Key Study Findings

- The NOAEL for fetuses was not established, as abortions occurred at all doses.
- Viable fetuses were not examined for visceral or skeletal findings.

Methods

Doses:	0, 10, 30 and 60 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Distilled water
Species/Strain:	Rabbit/Kbl:JW
Number/Sex/Group:	7 pregnant females/group
Age:	Females 18-19 weeks
Weight:	Females: 3.07-3.66 kg on the day of successful copulation
Study design:	Y-32720 was administered daily from Days 6-18 of pregnancy

Observations and Results for Dams

Mortality

One dam at the HD died on pregnancy Day 21 after a decrease in food consumption from pregnancy Day 7 and emaciation from pregnancy Day 13. A dosing error was suggested because of trachea and lung findings of foamy fluid.

Clinical Signs

Observations were performed once on Day 0 and daily during pregnancy on Days 5-28.

Fecal volume was decreased in all treated groups. Other signs included loose stool in 1 dam at the LD and soiled fur in 1 at the MD and 1 at the HD.

Body Weight

Body weight recordings were taken once on Day 0, daily during pregnancy Days 6-19 and on Days 22, 25 and 28.

Decrease in body weight was observed in dams at the MD and HD, with an 11.6% decrease recorded at the HD on Day 22.

Food Consumption

Food consumption recordings were taken once on Day 0 and daily during pregnancy Days 6-19 and on Days 22, 25 and 28.

The Sponsor excluded the data from evaluation as dams spilled feed. However, a decrease in food consumption occurred in all treated dams from Day 7 on.

Necropsy
On Day 28 of pregnancy, dams were sacrificed and visceral organs examined macroscopically. The ovary and uterus were removed for fertility observations, the heart, liver, lungs, kidneys, spleen, adrenals and ovary were removed and weighed and the thymus, stomach, heart, lungs, liver, kidneys, adrenals, spleen, uterus, ovary and placentas of all control F were fixed in 10% neutral buffered formalin.

Eight treated dams aborted, with all dams having splenic atrophy, pinpoints on the lungs and hemorrhage of the thymus. Spleen weight was decreased at all doses and ovary weight at the HD; adrenal weight was increased at all doses and heart weight at the HD.

Cesarean Section Data
Embryos and fetuses were classified into implantation sites, resorptions, placental remnants, macerated fetuses, dead fetuses and viable fetuses.

Abortions occurred in all dosed groups with all fetuses dead in the affected litters. These occurred on pregnancy Day 20 for 2 dams at the LD, on pregnancy Days 20-24 for 6 dams at the MD and on pregnancy Day 21 and 24 for 2 dams at the HD. All dams at the LD that did not abort had live fetuses, but with increased post-implantation loss. Dams at the MD and HD had total post-implantation loss and corpora lutea were decreased in dams at the HD.

Offspring (Malformations, Variations, etc.)
Fetuses and placenta were weighed and approximately one-half of the fetuses fixed in ethyl alcohol and stained with alizarin red S (Dawson's method). The other half were preserved in 10% neutral buffered formalin and fixed in Bouin's solution. However, no visceral or skeletal examination was performed on fetuses.

External observations: There were no external findings in viable fetuses. However, 1 fetus at the LD had ascites and a thick, edematous placenta.

Visceral findings: Not performed.

Skeletal findings: Not performed.

Study title: An oral embryo-fetal development study in rabbits

Study no:	PCS-r987117
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Novartis Pharmaceutical Corporation, (b) (4) East Hanover, New Jersey, USA
Date of study initiation:	16 December 1998
GLP compliance:	Yes (USA and Switzerland, 1986)
QA statement:	Yes (Dated: 6 August 1999)
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100% purity

Key Study Findings

- The NOAEL for the dams was 1.5 mg/kg/day.
- The NOAEL for fetuses was 0.5 mg/kg/day, as there was a significant increase in embryo-fetal mortality at 1.5 and 5 mg/kg/day.
- There were observations of visceral and skeletal malformations in the 1.5 and 5 mg/kg/day fetuses.

Methods

Doses:	0, 0.5, 1.5 and 5 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Purified water
Species/Strain:	Rabbit/New Zealand White, Hra:(NZW)SPF
Number/Sex/Group:	20 pregnant females/group
Satellite groups:	3 for control females and 5 females/dose group
Age:	At least 23 weeks at dose initiation
Weight:	3.07-4.28 kg at dose initiation
Study design:	FTY720 was administered daily on gestation Days 7-20

Observations and Results for Dams

Mortality

Observations were performed twice daily on weekdays and once daily on weekends and holidays.

There was no dam mortality.

Clinical Signs

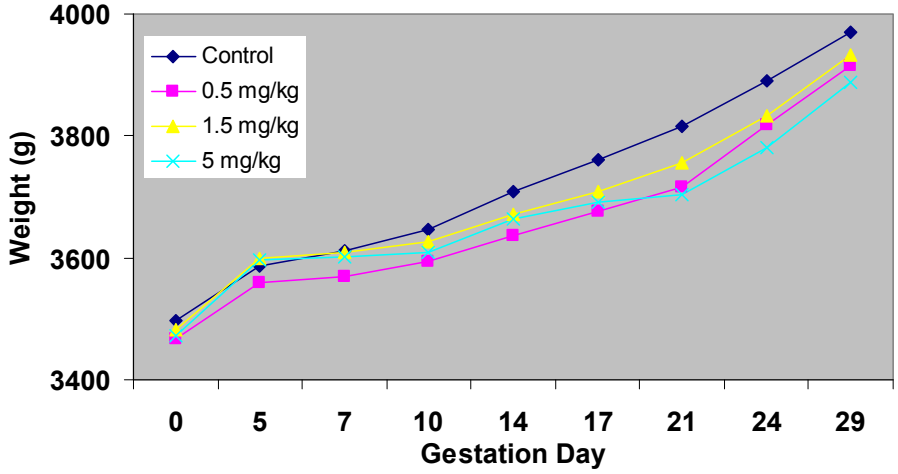
Observations were performed twice daily during treatment, pre-dose and within 3 hours post-dose.

Clinical signs observed included 1 dam with diarrhea at the LD, 1 dam with no stool at the MD on gestation Day 29, hair loss and decreased stools in all groups.

Body Weight
Body weight recordings were taken on gestation Days 0, 5, 7, 10, 14, 17, 21, 24 and 29.

There was a dose-related decrease in body weight change. However, this is reflected in a decrease in uterine weight at necropsy.

Graph plotted from body weight data



Food Consumption
Food consumption recordings were taken daily during gestation Days 5-29.

There were no food consumption changes observed.

Toxicokinetics:
Maternal blood samples were taken from satellite animals on gestation Day 20 pre-dose and at 2, 5, 10 and 24 hours post-dose. Fetuses were stored for tissue analysis of FTY720.

FTY720 was detected in one 2-hour control blood sample, but not detected in the fetal tissue. Exposure to FTY720 was greater than dose-proportional. Fetuses were exposed to FTY720 approximately dose-proportionally.

Table of maternal toxicokinetic data for FTY720 (Sponsor's)

Dose [mg/kg/day]	0.5		1.5		5	
Number of animals	5		4		4	
	Mean or Range	CV [%]	Mean or Range	CV [%]	Mean or Range	CV [%]
t_{max} [h]	0 - 5	*	0 - 2	*	2 - 2	*
C_{max} [ng/mL]	3.07	68	5.48	27	42.59	39
C_{max}/dose (1)	6.9	67	4.1	27	10	39
AUC (0-24h) [h·ng/mL]	16	40	57	11	273	30
AUC (0-24h)/dose (2)	36	40	43	11	61	30

(1): [(ng/mL)/(mg/kg/day)]
(2): [(h·ng/mL)/(mg/kg/day)]
CV: coefficient of variation: 100%·standard deviation / mean
*: not available or applicable

Table of fetal tissue concentration of FTY720 (Sponsor's)

Dose [mg/kg/day]	0.5	1.5	5
Number of animals	5	4	4
FTY720 [ng/g]	4.03	15.75	74.80
Ratio*	17	17	23

* Ratio of fetal tissue concentration to maternal blood concentration

Stability and Homogeneity.
FTY720 was considered stable; data were presented for dose Day 1 only. The Sponsor stated that "*Formulation uniformity testing was not required because FTY720 was formulated as solution*".

Necropsy
On gestation Day 29, dams were sacrificed and major visceral organs, including placentas examined macroscopically. The ovary, oviducts and uterus and contents were removed and weighted.

Uterus weight was dose-dependently decreased compared to control values by 3%, 7% and 40% at the LD, MD and HD, respectively. Ovarian cysts were observed in 1 dam at the MD and 1 at the HD.

Cesarean Section Data
Reproductive parameter were evaluated; corpora lutea, implantation sites, resorptions, dead fetuses and viable fetuses.

There was a dose-dependent increase in resorptions, 90% and 948% at the MD and HD, respectively; most were early resorptions. This resulted in a decrease in the viable fetuses; 4.3% and 44.7% at the MD and HD, respectively. There was also a non-statistically significant dose-dependent decrease in mean fetal weight of 1.9%, 2.8% and 4.9% at the LD, MD and HD, respectively.

Offspring (Malformations, Variations, etc.)
Fetuses were weighed and eviscerated fetuses stained with alizarin red.

External observations: There were several malformations and variations in fetuses at the HD, including cutis aplasia in 1 fetus, omphalocele in another, a muscle defect. Two fetuses from the same litter had malrotated hindlimbs, one of these also had the variation hyperflexion of the forepaws that was also observed in another pup from a different litter. Hyperflexion of the forepaws was observed in 1 fetus at the MD. Malformations observed in control fetuses included a protruding tongue and short snout in 1 fetus.

Visceral findings: There were several malformations and variations in fetuses at the MD and HD including an absent gallbladder in 2 at the MD and 1 at the HD. All groups had observations of small gallbladder; although the incidences at the HD were increased and in 1 litter all fetuses displayed the variation.

Table of visceral observations (Sponsor's)

	DOSAGE	0 MG/ KG/DAY	0.5 MG/ KG/DAY	1.5 MG/ KG/DAY	5.0 MG/ KG/DAY
Litters Evaluated	N	19	19	20	19
Fetuses Evaluated	N	158	160	159	92
Live	N	158	160	159	92
Dead	N	0	0	0	0
GALLBLADDER-ABSENT					
Fetal Incidence	N	0	0	2	1
	%	0.0	0.0	1.3	1.1
Litter Incidence	N	0	0	2	1
	%	0.0	0.0	10	5.3
TOTAL FETAL VISCERAL	MALFORMATIONS				
Fetal Incidence	N	0	0	2	1
	%	0.0	0.0	1.3	1.1
Litter Incidence	N	0	0	2	1
	%	0.0	0.0	10	5.3

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

	DOSAGE	0 MG/ KG/DAY	0.5 MG/ KG/DAY	1.5 MG/ KG/DAY	5.0 MG/ KG/DAY
Litters Evaluated	N	19	19	20	19
Fetuses Evaluated	N	158	160	159	92
Live	N	158	160	159	92
Dead	N	0	0	0	0
GALLBLADDER-SMALL					
Fetal Incidence	N	2	1	2	4
	%	1.3	0.6	1.3	4.3
Litter Incidence	N	1	1	2	2
	%	5.3	5.3	10	11
GALLBLADDER-ENLARGED					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	5.3	0.0	0.0	0.0
TOTAL FETAL VISCERAL	VARIATIONS				
Fetal Incidence	N	3	1	2	4
	%	1.9	0.6	1.3	4.3
Litter Incidence	N	2	1	2	2
	%	11	5.3	10	11

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

Skeletal findings: There were several malformations and variations 1 fetus at the LD had a misaligned sternebra, 1 fetus at the MD had thoracic malformations that included hemivertebra, fused centrum and misalignment centrum. At the HD 3 fetuses had thoracic malformations that included hemivertebra, fused centrum, misalignment, malpositioned vertebra, fused vertebra and absent vertebra, 2 fetuses had rib malformations including fused ribs, absent ribs and the third had a misshapen clavicle. Skeletal malformations were observed in 1 fetus from 1 litter at the MD and 5 fetuses from 2 litters at the HD.

Increases in variations in fetuses at the MD and HD included bent or delayed ossification of hyoid bone, misshapen sternebra, unossified metacarpal and phalanges.

There were 3 fetuses at the MD, from 2 different litters, with visceral or skeletal malformations and 5 at the HD, from 2 different litters.

Table of skeletal observations (Sponsor's)

	DOSAGE	0 MG/ KG/DAY	0.5 MG/ KG/DAY	1.5 MG/ KG/DAY	5.0 MG/ KG/DAY
Litters Evaluated	N	19	19	20	19
Fetuses Evaluated	N	158	160	159	92
Live	N	158	160	159	92
Dead	N	0	0	0	0
CLAVICLE-MISSHAPEN					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
STERNEBRA-MISALIGNED					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.6	0.0	1.1
Litter Incidence	N	0	1	0	1
	%	0.0	5.3	0.0	5.3
RIB-FUSED					
Fetal Incidence	N	0	0	0	3*
	%	0.0	0.0	0.0	3.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
RIB-ABSENT(UNILATERAL)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
THORACIC CENTRUM-FUSED					
Fetal Incidence	N	0	0	1	1
	%	0.0	0.0	0.6	1.1
Litter Incidence	N	0	0	1	1
	%	0.0	0.0	5.0	5.3
THORACIC CENTRUM-MISALIGNED					
Fetal Incidence	N	0	0	1	2
	%	0.0	0.0	0.6	2.2
Litter Incidence	N	0	0	1	1
	%	0.0	0.0	5.0	5.3
THORACIC CENTRUM-FUSED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
THORACIC CENTRUM-HEMICENTRIC					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
THORACIC HEMIVERTEBRA					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.0	0.0
THORACIC VERTEBRA-MALPOSITIONED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
THORACIC VERTEBRA-FUSED					
Fetal Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	2.2
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
THORACIC VERTEBRA-ABSENT (UNILATERAL)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
LUMBAR CENTRUM-MISALIGNED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	1	1	5**
	%	0.0	0.6	0.6	5.4
Litter Incidence	N	0	1	1	2
	%	0.0	5.3	5.0	11

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

9.3 Prenatal and Postnatal Development

Study title: An oral pre- and postnatal development study in rats

Study no:	PCS-r997110
Study report location:	4.2.3.5.3
Conducting laboratory and location:	Novartis Pharmaceutical Corporation, (b) (4), East Hanover, New Jersey, USA
Date of study initiation:	21 Sept 1999
GLP compliance:	Yes (USA)
QA statement:	Yes (Dated 14 Jul 2000)
Drug, lot #, and % purity:	FTY720, Batch № 98905, 100% purity

Key Study Findings

- Reproductive changes for F_0 dams included a slight decrease in corpora lutea and an increase in pre-implantation loss. The number of live fetuses was dose-dependently reduced.
- Dose-dependent increase in stillborn F_1 pups from several dams administered 0.5 mg/kg/day.
- Two litters from dams at 0.5 mg/kg/day died by Day 4 and there was a dose-dependent decrease in surviving pups (increase in missing pups) during the first week. F_1 pup survival rates to 21 days postpartum were significantly decreased; 96%, 93% and 62.5% for 0.05, 0.15 and 0.5 mg/kg/day, respectively.
- The NOAEL for dams was 0.15 mg/kg/day.
- The NOAEL for fetuses was not established, as survival rate was decreased at all doses.

Methods

Doses:	0.05, 0.15 and 0.5 mg/kg
Frequency of dosing:	Daily
Dose volume:	4 ml/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Purified Water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	25 Pregnant females/group
Age:	11 weeks at arrival
Weight:	203-247 kg on gestation Day 0
Deviation from study protocol:	FTY720 was administered daily from gestation Days 6 to lactation Day 20

Observations and Results for F_0 Dams

Mortality

Observations were performed twice daily on weekdays and once daily on weekends and holidays.

There were no unscheduled mortalities dams during the study. However, 2 F at the HD were sacrificed early during the lactation period as their litters died.

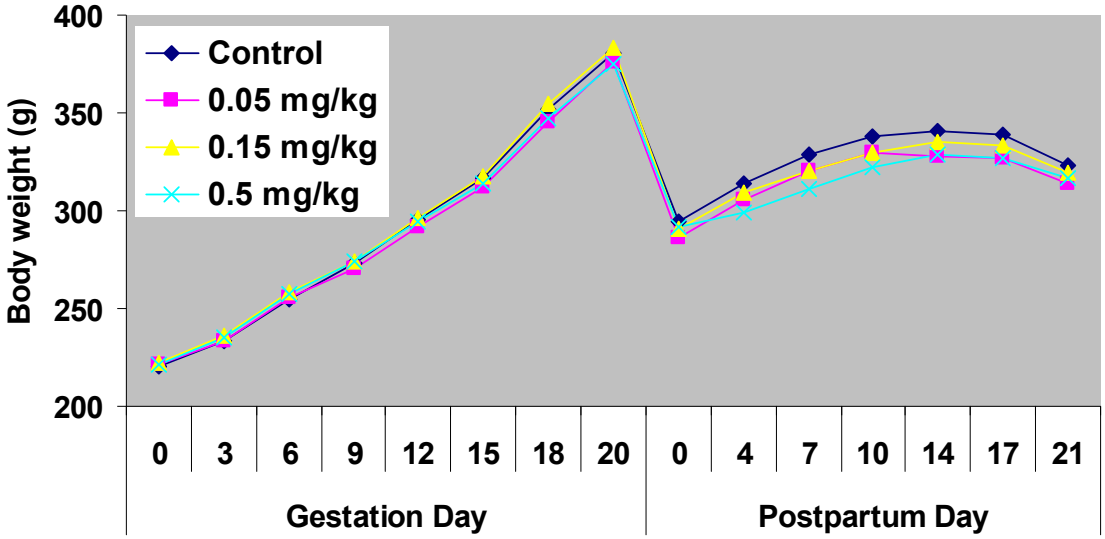
Clinical Signs
Observations were performed once daily pretreatment, twice daily during treatment, pre-dose and within 3 hours post-dose.

The major clinical signs included material around the eye and hair loss observed in all groups.

Body Weight
Body weight recordings for F_0 dams were taken during gestation on Days 0, 3, 6, 9, 12, 15, 18 and 20 and during lactation on Days 0, 4, 7, 10, 14, 17 and 21.

No treatment changes in body weight occurred during gestation. From lactation Day 0-7, there was a significant reduction in body weight at the HD.

Graph of body weight



Food Consumption
Food consumption for F_0 dams were recorded during gestation on Days 3, 6, 9, 12, 15, 18 and 20 and during lactation on Days 0, 4, 7, 10 and 14.

Food consumption for dams at the HD was significantly decreased during gestation Days 9-12 and postpartum Days 0-14.

Reproduction parameters
The number of implantation sites was assessed.

There was a slight decrease in corpora lutea (means: 18.9, 17.3, 17.9 and 17.4) and an increase in pre-implantation loss (means: 17.1, 16.1, 16.3 and 15.9) for dams at control,

LD, MD and HD, respectively. The number of live fetuses was 365, 352, 341 and 299 at the control, LD, MD and HD, respectively. See F_1 mortality and clinical signs section below for further comments.

Necropsy
Necropsy occurred on gestation Day 25 for F_0 animals that did not deliver and on lactation Day 21 for F_0 F. Culled pups were sacrificed on lactation day 21 and all major viscera evaluated grossly; F_0 animals were then discarded.

There were no remarkable findings.

Observations and Results for F_1 Generation

Mortality and Clinical signs
Pre weaning
Observations were performed on all pups from postpartum Days 0-21.

There were 1, 1, 3 and 7 stillborn pups from 1, 1, 1 and 6 dams at control, LD, MD and HD, respectively. Two entire litters from dams at the HD died during the first 4 days of the lactation period. There was a dose-dependent increase in missing pups over the first week of lactation of 1, 5, 16 and 64 pups at the control, LD, MD and HD, respectively. This resulted in a significant decrease in pup survival rates to 21 days postpartum of 96%, 93% and 62.5% at the LD, MD and HD, respectively; the control survival rate was 99%. The reason for mortality was undetermined. Hematomas were observed in 1 pup from each control, MD and HD groups. Two, 1 and 5 pups were pale at the LD, MD and HD, respectively. A pup at the HD had an umbilical hernia.

Post-weaning
Observations for mortality were performed twice daily on weekdays and daily on weekends and holidays. Clinical signs observations were performed daily.

The major clinical signs included wounds of cuts, scratches and scabs and hair loss observed in all groups and material round the eye in M at the HD and F at the MD.

Body Weight
Body weight recordings for F_1 M were taken weekly on Days 28, 35, 42 etc. until necropsy. F_1 F body weights were taken weekly and, if selected for fertility, on gestation Days 0, 3, 6, 9 and 13.

Pre-weaning: Pups at the LD and MD had a 5-7% decrease in body weight; in contrast, the surviving pups at the HD had a 1-8% increase in body weight.

Post-weaning: The changes observed pre-weaning were continued post-weaning with slight decreases in M and F at the LD and MD and increase at the HD.

F_1 gestation: Similar changes were observed during gestation, with slight decreases in M and F at the LD and MD and increase at the HD.

Physical development
Observations and tests were performed on all animals for righting reflex on Days 0-2, pinna detached on Days 2-5, eye opening on Days 14-17, vaginal opening on Days 32-40, preputial separation on Days 42-48, open field motor activity on Days 56-58, acoustic startle on postpartum Day 28 and pupillary reflex on Day 35.

There was no difference in righting reflex, pinna detached, eye opening, acoustic startle and papillary reflex in F_1 animals, preputial separation in M and vaginal opening in F.

Neurological assessment
Tests were performed on 1/sex/litter, for passive avoidance (learning/acquisition) on postpartum Days 63±2, passive avoidance (retention/memory) on Days 70±2, M-maze (learning/acquisition) on Days 63±2, Morris Maze (retention/memory) on Days 70±2.

In the open field test, there was an increase in central and decrease in peripheral beam breaks in M at the MD and HD. However, the total number of breaks was similar suggesting no change in activity. In F at the MD and HD, there was only a decrease in peripheral beam breaks with no change in central beam breaks resulting in a decrease in total breaks suggesting a decrease in activity, but the effect was not dose-dependent.

There were no changes in M acquisition/learning phase of the passive avoidance evaluation. In the retention/memory phase, there was a statistically significant decrease in the number of M at the HD reaching the criterion. In F at the HD, there was a slight increase in the number of trials to reach the acquisition/learning phase criterion and a decrease in the number of animals reaching the criterion. In addition, there was a slight increase in the number of trials to reach the retention/memory phase criterion. When differences between the acquisition and retention phases were assessed, there was an increase in total latency time/number of trials completed in F at all doses; however, these changes were not statistically significant. In the Water maze test, M showed no differences; however, the number of F at the HD reaching criterion was decreased.

Reproduction
For each pregnant F_1 F sacrificed, the number of corpora lutea, implantation sites, live fetuses and early resorptions were assessed.

There were no changes in the reproductive parameters of corpora lutea, implant sites, resorption and live fetuses in the F_1 dams (see table below).

Table of mean F_1 dam reproductive parameters

	Dam				Resorptions		Fetuses	
Dose	**Mated**	**Pregnant**	**C. lutea**	**Implant sites**	**Early**	**Late**	**Dead**	**Live**
Control	24*	23	18.9	17.1	1.2	0	0	365
0.05 mg/kg/day	24	23	17.3	16.1	0.8	0	0	352
0.15 mg/kg/day	25	22	17.9	16.3	0.8	0	0	341
0.5 mg/kg/day	23	20	17.4	15.9	1.0	0	0	299

*** 1 female sacrificed moribund**

Necropsy
Animals were sacrificed on lactation Day 21 for culled pups and pups not selected for F_1 fertility assessment, gestation Day 13 for F_1 F with positive evidence of mating, 11 days after mating for non-pregnant F_1 F and after mating for F_1 M. All stillborn F_1 pups or pups found dead between postpartum Days 0-4 heart and major vessels were examined. All other F_1 animals were discarded after gross evaluation.

After weaning (lactation Day 21): There were few findings observed. The primary observation was short renal papilla that was seen in pups from all dose groups. Most of the dead pups were discarded due to autolysis.

After fertility assessment (lactation Day 21) There were few findings observed. The primary observation was short renal papilla, observed in animals from all groups.

Stability and Homogeneity
FTY720 was chemically stable for at least 40 days at 6°C and at least 4 hours at room temperature. Samples were taken from target solutions of 0.01, 0.05 and 2 mg/ml at different time points that included; initial, 4 hours stirring at room temperature, 21, 35 and 40 Days at 6°C. Stability data showed that the 0.01, 0.05 and 2 mg/ml solutions ranged between 93-109% of the target concentration after 4 hours at room temperature and ranged between 95-102% of the target concentration after 35-40 days at 6°C. The Sponsor stated that "*Formulation uniformity testing was not required because FTY720 was formulated as solution*".

10 Special Toxicology Studies

10.1 Impurities

Summary for Impurities and starting material
Several impurities have genotoxic structural alerts, these include: (b) (4) and degradation products (b) (4).
With the proposed daily dose of 0.5 mg (8.3 µg/kg) for chronic treatment, the levels of impurities must not exceed (b) (4). The following tables outline the found expected daily levels of impurities in FTY720.

Sponsor's table

Table 2-4 Impurities by HPLC found in Fingolimod Hydrochloride

Impurity	Set limit	Range found in development and foreseen market supplies	Results found in TOX1/FTY720 Tox mix batch 00/1
[redacted] (b) (4)			

The reporting limit for the impurities tested by HPLC is (b) (4)

*: in batch 0324029, (b) (4) of (b) (4) was found.

In all batches manufactured for market supplies all specified and unspecified impurities by HPLC have been found below (b) (4) so far.

Study title: TOX1/FTY720: Mutagenicity test using *Salmonella typhimurium* (batch control)

Study no.:	PCS-r0112007
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland.
Date of study initiation:	3 April 2001
GLP compliance:	Yes (Switzerland 1997)
QA statement:	Yes (Dated 28 Jun 2001)
Drug, lot #, and % purity:	TOX1/FTY720, batch 00/1 with 0.5% (b) (4) 0.7% (b) (4), 0.8% (b) (4), 0.6% (b) (4)

Key Study Findings:

- Inhibition of growth occurred at concentrations equal or greater then 25 µg, and there was no increase in revertant colonies observed in any strain.
- FTY720 (batch 00/1) is negative for reverse mutations in the concentration range tested and the Sponsor's conclusion that "TOX1/FTY*720 did not show evidence of a mutagenic potential*" appears appropriate.
- However, 2AA was the sole positive control for S-9 mix, with no further characterization of the batch performed.

Methods

Strains:	*S. typhimurium* TA1535, TA97a, TA98, TA100 and TA102.
Concentrations in definitive study:	6.25, 12.5, 25, 50 and 100 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Doses were selected form a previous (unspecified) experiments.
Negative control:	DMSO.
Positive controls	
Without S-9 mix:	3 µg Sodium azide for TA1535 and TA100, 100 µg 9-aminoacridine for TA97a, 3 µg benzo(a)pyrene for TA98 and mitomycin C for TA102.
With S-9 mix:	3 µg 2-aminoanthracene (2AA) for TA1535, TA98 and TA100, 10 µg 2AA for TA97a and TA102.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Revertant colonies were counted following 3 days of incubation at 37°C.

Study Validity

The plate incorporation, without pre-incubation, test was performed. 2AA was used as the positive control for all strains in the presence of S-9 mix, prepared from M Wistar rats treated with Aroclor 1254, and no data were presented to demonstrate that each batch of S-9 mix was characterized with a microsomal enzyme. Apart from the lack of further characterization of the S-9 mix, the study appears valid. Triplicates were performed for the study; the criteria for a positive result was that FTY720 (batch 00/1) would produce a mean increase of revertant colonies double that observed for the control plates in at least one concentration and one strain, apart from strain TA102 for which the increase is 1.5.

Results

Reduction of background growth and signs of cytotoxicity were observed in all *S. typhimurium* strains at FTY720 doses outlined in the table below. No increase in revertant colonies was observed in any strain at any dose of FTY720. Therefore, FTY720 (batch 00/1) is negative in the AMES assay and the Sponsor's conclusion that "TOX1/FTY*720 did not show evidence of a mutagenic potential*" appears appropriate.

Table of toxicity (Sponsor's)

strain:	TA1535		TA97a		TA98		TA100		TA102	
S9:	-	+	-	+	-	+	-	+	-	+
[µg/plate]	25	100	50	100	50	100	25	100	50	50

Study title: TOX2/FTY720: Mutagenicity test using *Salmonella typhimurium* (batch control)

Study no.:	PCS-r0350411
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland.
Date of study initiation:	5 December 2003
GLP compliance:	Yes (Switzerland 1997)
QA statement:	Yes (Dated 28 Jan 2004)
Drug, lot #, and % purity:	FTY720, batch 03/1 with 4.6% NAP (b) (4) 4.5% NAP (b) (4) 4.8% NAP (b) (4)

Key Study Findings:

- Inhibition of growth occurred at 50 µg/plate in TA100 +S-9 and at 100 µg/plate for all other conditions; there was no increase in revertant colonies observed in any strain.
- FTY720 (batch 03/1) was negative for reverse mutations in the concentration range tested and the Sponsor's conclusion that "TOX2/FT*Y720 did not show evidence of a mutagenic potential*" appears appropriate.

Methods

Strains:	*S. typhimurium* TA1535, TA97a, TA98, TA100 and TA102.
Concentrations in definitive study:	6.25, 12.5, 25, 50 and 100 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Doses were selected form a previous (unspecified) experiment.
Negative control:	DMSO.
Positive controls:	
Without S-9 mix:	3 µg Sodium azide for TA1535 and TA100, 100 µg 9-aminoacridine for TA97a, 2-nitrofluorene for TA98 and mitomycin C for TA102.
With S-9 mix:	3 µg 2-aminoanthracene (2AA) on TA1535, TA98 and TA100, 10 µg 2AA for TA97a and TA102, 3 µg benzo(a)pyrene for TA98
Formulation/Vehicle:	DMSO.
Incubation & sampling time:	Revertant colonies were counted following 3 days of incubation at 37°C.

Study Validity

The plate incorporation test was performed. The S-9 mix was prepared from M rats treated with Aroclor 1254. Triplicates were performed for the study; the criteria for a positive result was that FTY720 (batch 03/1) would produce a mean increase of revertant colonies double that observed for the control plates in at least one

concentration and one strain, apart from strain TA102 for which the increase is 1.5. The study appears valid.

Results

Reduction of background growth and signs of cytotoxicity were observed in all *S. typhimurium* strains at FTY720 doses of 100 µg/plate, except for TA100 +S-9 that was observed at 50 µg/plate. No increase in revertant colonies was observed in any strain at any dose of FTY720. Therefore FTY720 (batch 03/1) is negative in the AMES assay and the Sponsor's conclusion that "TOX2/FTY*720 did not show evidence of a mutagenic potential*" appears appropriate.

Study title: FTY720 (b) (4): Ames test

Study no.:	PCS-r0113138
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Unknown
Date of study initiation:	Unknown
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	FTY720 (b) (4), batch (b) (4), purity unknown

Key Study Findings:

- The test is invalid.

Methods

Strains:	*S. typhimurium* TA98 and TA100.
Concentrations in definitive study:	15, 50, 150, 500, 1,500 and 5,000 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Not stated.
Negative control:	DMSO.
Positive controls:	
Without S-9 mix:	Not stated
With S-9 mix:	Not stated
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Revertant colonies were counted following 3 days of incubation at an unstated temperature.

Study Validity

The study appears invalid, as only TA98 and TA100 strains of *S. typhimurium* were used. In addition, there is a lack of detail; the positive controls were not identified and no data were submitted.

Results

The Sponsor's concludes that "*FTY720 (b) (4) did not show evidence of a mutagenic potential*"; however it is impossible for the reviewer to confirm this statement.

Study title: FTY720 (b) (4) Ames test

Study no.:	PCS-r0113139
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Unknown
Date of study initiation:	Unknown
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	FTY720 (b) (4), batch (b) (4), purity unknown

Key Study Findings:

- The test was invalid.

Methods

Strains:	*S. typhimurium* TA98 and TA100.
Concentrations in definitive study:	15, 50, 150, 500, 1,500 and 5,000 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Not stated.
Negative control:	DMSO.
Positive controls:	
Without S-9 mix:	Not stated
With S-9 mix:	Not stated
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Revertant colonies were counted following 3 days of incubation at an unstated temperature.

Study Validity

The study appears invalid, as only TA98 and TA100 strains of *S. typhimurium* were used. In addition, there is a lack of detail; the positive controls were not identified and no data were submitted.

Results

The Sponsor's concludes that "*FTY720 (b) (4) did not show evidence of a mutagenic potential*", however it is impossible for the reviewer to confirm this statement.

Study title: FTY720 (b) (4): Ames test

Study no.:	PCS-r0113140
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Unknown
Date of study initiation:	Unknown
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	FTY720 (b) (4), batch (b) (4), purity unknown

Key Study Findings:

- The test was invalid.

Methods

Strains:	*S. typhimurium* TA98 and TA100.
Concentrations in definitive study:	15, 50, 150, 500, 1,500 and 5,000 µg per plate in the presence and absence of S-9 mix.
Basis of concentration selection:	Not stated.
Negative control:	DMSO.
Positive controls:	
Without S-9 mix:	Not stated
With S-9 mix:	Not stated
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Revertant colonies were counted following 3 days of incubation at an unstated temperature.

Study Validity

The study appears invalid, as only TA98 and TA100 strains of *S. typhimurium* were used. In addition, there is a lack of detail; the positive controls were not identified and no data were submitted.

Results

The Sponsor's concludes that "*FTY720* (b) (4) *did not show evidence of a mutagenic potential*", however it is impossible for the reviewer to confirm this statement.

Study title: TOX1/FTY720: Chromosomal aberration test with V79 Chinese hamster cells

Study no.:	PCS-r0112103
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland
Date of study initiation:	15 March 2001
GLP compliance:	Yes (Switzerland, 1997)
QA statement:	Yes (Dated 06 August 2001)
Drug, lot #, and % purity:	TOX1/FTY720, batch 00/1 with 0.5% (b) (4) 0.7% (b) (4), 0.8% (b) (4), 0.6% (b) (4)

Key Study Findings

- FTY720 (batch 00/1) is negative in the chromosomal aberration assay. However, the OECD guideline that 200 cells need to be examined was not met for every concentration in every condition. In the experiment (D), 3-hour incubation in the absence of S-9 the mitotic index was greater than 100% of control level.
- There was an increase in polyploidy in both the absence and presence of S-9.

Methods

Cell line:	V79 Chinese hamster cells
Concentrations in definitive study:	
Experiment B:	
Without S-9 mix, 20 h:	0.1, 0.2, 0.3, 0.4, 0.7, 1.1, 1.7, 2.8, 4.5 µg/ml
With S-9 mix, 3 h:	25, 27, 29, 31, 34, 36, 39, 42, 45 µg/ml.
Experiment D:	
Without S-9 mix, 3 h:	1, 1.4, 1.9, 2.5, 3.5, 4.7, 6.4, 8.8, 12 µg/ml.
Basis of concentration selection:	Selection of concentrations was done by examination of the cell counts during the chromosome aberration test. Experiments A and B were not valid due to lack of enough metaphases. Concentrations were given based on theoretical calculations.
Negative control:	1% DMSO
Positive control:	15 mM ethyl methanesulphonate and 17.5 µM Cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	20 hour incubations at 37°C in the absence of S-9 mix and 3 hour incubation with S-9 mix followed by an addition 17 hours after removal of S-9 mix

Study Validity

The study is not completely valid. In experiment D, the OECD guidelines indicate that the mitotic index should be reduced by greater than 50%; at the analyzed concentrations, the MI was greater than 100% of control levels. Also, the need for 200 cells to be examined was not met.

Results

In experiment B (20 hours), the highest of the 3 concentrations assessed in the absence of S-9, 2.8 µg/ml (mitotic index was -72.1%), had 6.4% of cells with aberrations compared to the positive control of 14%, in the presence of S-9, no increase in aberrations was observed, but concentrations assessed had decreased cell growth. In experiment D (3 hours) in the absence of S-9 there was no increase in aberrations observed, but all 3 concentrations assessed had mitotic indices greater than 100% of control level, with decreased cell growth. In addition, the need for 200 cells to be examined was not always met. There was an increase in the number of polyploidy cells (5.4-22.2%) in both the absence and presence of S-9.

Study title: TOX2/FTY720: Chromosomal aberration test with cultured human peripheral blood lymphocytes

Study no.:	PCS-r0350413
Study report location:	4.2.3.7.6.1
Conducting laboratory and location:	Novartis Pharma AG, Basal, Switzerland.
Date of study initiation:	24 November 2003
GLP compliance:	Yes (Switzerland, 1997)
QA statement:	Yes (Dated 21 April 2004)
Drug, lot #, and % purity:	FTY720, batch 03/1 with 4.6% NAP (b) (4) 4.5% NAP (b) (4) 4.8% NAP (b) (4)

Key Study Findings

- FTY720 (batch 03/1) did not increase chromosomal aberrations or polyploidy in any conditions. Therefore, the Sponsor's conclusion that "*TOX2/FTY720 did not show any clastogenic potential*" appears appropriate.

Methods

	Cell line:	Human lymphocytes
Concentrations in definitive study:		
Experiment A	With S-9 mix, 3 h:	18.2, 20.1, 22.3, 24.7, 27.3, 30.3, 33.5, 37.1, 41.1, 45.5 µg/ml
	Without S-9 mix, 3 h:	0.91, 1.23, 1.66, 2.24, 3.03, 4.09, 5.53, 7.47, 10.09, 13.64 µg/ml.
	Without S-9 mix, 20 h:	0.09, 0.14, 0.23, 0.36, 0.56, 0.88, 1.39, 2.2, 3.46, 5.45 µg/ml
Experiment B	With S-9 mix, 3 h:	3, 3.7, 4.6, 5.6, 7, 8.6, 10.6, 13.1, 16.2, 20 µg/ml
	Without S-9 mix, 20 h:	1, 1.4, 1.8, 2.5, 3.3, 4.5, 6.1, 8.2, 11.1, 15 µg/ml
Experiment C		
	Without S-9 mix, 20 h:	2, 2.5, 3.2, 4, 5, 6.3, 8, 10.1, 12.7, 16 µg/ml
	Basis of concentration selection:	The highest concentration selected for analysis was a concentration with a mitotic index between 30-50% of the corresponding vehicle control.
	Negative control:	DMSO
	Positive control:	5 and 13.1 mM ethyl methanesulphonate and 55 µM Cyclophosphamide
	Formulation/Vehicle:	DMSO
	Incubation & sampling time:	20 hour incubations at 37°C in the absence of S-9 mix and 3 hour incubation with S-9 mix followed by an addition 17 hours after removal of S-9 mix

Study Validity

Although some parts of experiments were invalid, the overall study was valid as TOX2/FTY720 was investigated with 3 hour incubation in the absence and presence of S-9 and incubated for 20 hours in the absence of S-9.

Results

Experiment A slides were not analyzed for –S-9 20 hour incubation and +S-9 3 hour incubation due to lack of adequate toxicity. Poor quality metaphases in the -S-9 20 hour incubation part of experiment B rendered it invalid. Therefore, valid experiments were experiment A, 3 hour incubation in the absence of S-9, experiment B, 3 hour incubation in the presence of S-9 and experiment C, incubation for 20 hours in the absence of S-9.

In all experiments A (3 hours, -S-9), B (3 hours, +S-9) and C (20 hours, -S-9) there was no increase in aberrations or polyploidy.

Study title: FTY720 (b) (4): Micronucleus test *in vitro* using mouse lymphoma (L5178Y) cells.

Study no.:	PCS-r0114089
Study report location:	4.2.3.7.6
Conducting laboratory and location:	Unknown
Date of study initiation:	Unknown
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	FTY720 (b) (4), Batch № (b) (4), purity unknown.

Key Study Findings

- The test was invalid.

Methods

Cell line:	L5178Y mouse lymphoma cells
Concentrations in definitive study:	
Experiment 1	
20 hour treatment without S-9 mix:	1,078.1, 1,798.5 and 3,000 µg/ml
3 hour treatment with S-9 mix:	1,078.1, 1,798.5 and 3,000 µg/ml
Experiment 2	
20 hour treatment without S-9 mix:	1,426.2, 1,888.3 and 2,500 µg/ml
3 hour treatment with S-9 mix:	1,426.2, 1,888.3 and 2,500 µg/ml
Basis of concentration selection:	Unknown
Negative control:	DMSO
Positive control:	
Without S-9 mix:	Cyclophosphamide, 50 µM
With S-9 mix:	Ethyl-methanesulphonate, 4 mM
Formulation/Vehicle:	DMSO
Incubation & sampling time:	For the main test treatment with S-9 mix was for 3 hours and sampling was performed after 24 hours after 3 hour incubation or 48 hours after the start of 20 h treatment.

Study Validity

Certain information was missing and no raw data were presented. The study was also not performed under GLP or quality assured.

Results

The Sponsor's concludes that "*FTY720* (b) (4) *was neither clastogenic nor aneugenic under the test conditions used*", however it is impossible to confirm this statement.

Study title: 4-Week oral (gavage) impurity toxicity study in the rat

Study no.:	PCS-r017020
Study report location:	EDR: 4.2.3.7.6.1
Conducting laboratory and location:	Novartis, East Hanover, New Jersey, USA.
Date of study initiation:	6 February 2001
GLP compliance:	Yes (Switzerland 2000)
QA statement:	Yes (Dated: 16 July 2001)
Drug, lot #, and % purity:	*TOX1/FTY720, Batch № 00/1, 97.3% with 0.5% (b) (4), 0.7% (b) (4), 0.8% (b) (4), 0.6% (b) (4) and 0.2% other related substances and FTY720, Batch № 9922006, 99.9%

Key Study Findings

- Lymphocytes were reduced in all treated groups.

- Histopathological findings included atrophy of the lymphoid organs and smooth muscle hypertrophy, collagen and increased macrophages in the lungs, that were more severe in TOX1/FTY720 treated animals.
- The groups treated with TOX1/FTY720 appeared to have a slight increase in the severity of toxic effects.
- These data suggest that degradation products (b) (4) at the levels used slightly exacerbate the toxicities seen with FTY720.

Methods

Doses:	0, 1#, 10#, 1.03* and 10.3* mg/kg/day see above (Drug) for * and # see table below for equivalent doses for impurities
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Rat/IGS Wistar
Number/Sex/Group	
Main study:	10
Age:	Approximately 8 weeks at start of treatment
Weight	
Males:	213.2-247.6 g
Females:	156.3-185.1 g
Deviation from study protocol:	The FTY720 HD animals (Group 3) had no ophthalmoscopic examination

Table 3.3-2. Equivalent doses for components of formulations for Groups 2 – 5

		Component, equivalent dose and percent of total				
Group	Dose mg/kg/day	FTY720 mg/kg/day (% of total)	541-00 mg/kg/day (% of total)	542-00 mg/kg/day (% of total)	543-00 mg/kg/day (% of total)	544-00 mg/kg/day (% of total)
2	1.0	1 (99.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
3	10	10 (99.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
4	1.03	1 (97.3%)	0.005 (0.5%)	0.007 (0.7%)	0.008 (0.8%)	0.006 (0.6%)
5	10.3	10 (97.3%)	0.05 (0.5%)	0.07 (0.7%)	0.08 (0.8%)	0.06 (0.6%)

Observations and Results

Mortality

Observations were made twice daily and at least once daily at weekends and plant closings.

One FTY720 F at the LD died on Day 29 and was considered to be related to the bleeding/anesthesia procedures.

Clinical Signs
Observations were performed at least daily during pre-treatment and weekly during treatment.

There were no drug-related changes, with only occasional fur thinning/loss.

Body Weights
Body weights were recorded once during pre-treatment, weekly during treatment and at necropsy.

There was a slight decrease in body weight gain over the course of treatment that at Day 29 was 14% for M and 16% for F at the HD. Animals treated with TOX1/FTY720 had a greater decrease in body weight gains with decreases at Day 39 of 21% for M and 28% for F at the HD.

Table of body weight gains

	Dose	Day 8				Day 29			
Group	(mg/kg/day)	Male		Female		Male		Female	
Control		30.9 g	0%	15.4 g	O%	97.9 g	0%	41.8 g	0%
FTY720	1	27.6 g	-11%	10.4 g	-32%	79.8 g	-18%	36.6 g	-12%
FTY720	10	24.2 g	-22%	7.4 g	-52%	84.5 g	-14%	35.2 g	-16%
TOX1/FTY720	1.03	30.1 g	-3%	12.0 g	-22%	88.4 g	-10%	36.8 g	-12%
TOX1/FTY720	10.3	21.0 g	-32%	3.3 g	-79%	77.6 g	-21%	30.1 g	-28%

Food Consumption
Food consumption was recorded weekly during treatment.

There was a decrease in food consumption over the course of treatment for HD groups, but was greatest over the first week (see table below).

Table of food consumption

	Dose	Day 8				Day 29			
Group	(mg/kg/day)	Male		Female		Male		Female	
Control		24.9 g	0%	19.6 g	O%	101.4 g	0%	77.4 g	0%
FTY720	1	24.8 g	0%	18.1 g	8%	96.8 g	5%	74.9 g	3%
FTY720	10	21.9 g	12%	17.5 g	11%	93.4 g	8%	75.9 g	2%
TOX1/FTY720	1.03	24.6 g	1%	18.3 g	7%	98.5 g	3%	74.0 g	4%
TOX1/FTY720	10.3	21.1 g	15%	15.4 g	21%	90.7 g	11%	68.4 g	12%

Ophthalmoscopy
Examinations were performed pre-treatment on all animals and on Day 24 for groups 1 and 5 animals.

The Sponsor stated that "*ophthalmoscopic examinations did not reveal any ocular changes attributable to treatment with TOX1/FTY720*"; however no raw data were submitted for review.

Hematology

Blood samples were taken in Week 5. The following parameters, listed in the study report, were assessed.

erythrocytes	Wintrobe indices	white blood cell count
hematocrit	red cell distribution width (RDW)	white blood cell differential
hemoglobin	reticulocytes	platelets

All treatment groups had a decrease in WBCs. M and F lymphocytes were decreased (89-93%) in all treatment groups, neutrophils were increased in M at the HD (45-62%) and monocytes were dose-dependently increased in M dosed with FTY720 (106% and 132% at the LD and HD, respectively) and TOX1/FTY720(93% and 164% at the LD and HD, respectively). In F, monocytes were decreased (38-69%).

Clinical Chemistry

Blood samples were taken in Week 5. The following parameters, listed in the study report, were assessed.

alanine aminotransferase	globulins (G)	chloride
alkaline phosphatase	glucose	calcium
aspartate aminotransferase	urea	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	sodium	cholesterol
albumin (A)	potassium	A/G ratio

In M, statistically significant increases in alanine aminotransferase and potassium were observed at the TOX1/FTY720 HD and a trend for increase at the FTY720 HD.

In F, increase in alanine aminotransferase, phosphorus and cholesterol was observed at the TOX1/FTY720 HD and at the FTY720 HD. Cholesterol was statistically increased at the FTY720 LD. In general, there were no major differences between the 2 FTY720 batches.

Urinalysis

Urine samples were taken in Week 4. The following parameters, listed in the study report, were assessed.

specific gravity	glucose*	protein*
bilirubin*	ketones*	urobilinogen*
blood*	pH*	

*test strip determinations

There were no treatment related changes.

Gross Pathology
Gross pathology was assessed in all animals.

The macroscopic findings were generally unremarkable. There were TOX1/FTY720 animals that had small spleens.

Organ Weights
At necropsy, the organs listed in the table below, in the histopathology section, from the study report, were weighed.

In M, the relative weight of heart (7%, 15%, 10% and 17%) was increased and spleen (22%, 29%, 24% and 24%) decreased. In F, the relative weight of brain (1%, 6%, 1% and 7%), thyroid (27%, 44%, 33% and 33%) and heart (9%, 16%, 9% and 18%) was increased and spleen decreased (22%, 18%, 17% and 16%). Values in parenthesis are LD and HD FTY720 and LD and HD TOX1/FTY720, respectively.

Lungs were not weighed. There were no major differences between TOX1FTY720 and FTY720 treated M and F.

Histopathology
At necropsy the following organs, listed in the study report, were prepared for histopathology examination. Tissues were fixed in 10% formalin, except for testes and epididymides that were fixed in Bouin's solution and eyes and Harderian glands that were fixed in 3% Sorensen's buffered glutaraldehyde.

Tissue list for collection, weighing (W) and/or processing (P)

W	P	adrenal	W	P	ovary
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
W	P	brain	W	P	pituitary
	P	cecum	W	P	prostate
	P	cervix		P	rectum
	P	colon		P	salivary gland
	P	duodenum		P	sciatic nerve
	P	epididymis		P	seminal vesicle
	P	esophagus		P	skeletal muscle
	P	eye		P	skin
	P	femur/tibia		P	spinal cord
	P	harderian gland	W	P	spleen
W	P	heart		P	sternum
	P	Ileum		P	stomach
	P	Jejunum	W	P	testis
W	P	Kidney	W	P	thymus
	P	Lacrimal gland	W	P	thyroid
W	P	Liver		P	tongue
	P	Lung		P	trachea
	P	Lymph node - bronchial		P	urinary bladder
	P	Lymph node - mandibular	W	P	uterus
	P	Lymph node - mesenteric		P	vagina
	P	Mammary gland		P	macroscopic lesions
		Nasal passage			animal identification

Adequate Battery: Yes
Peer Review: Yes. All assessments were peer-reviewed per SOP

The main histological findings were atrophy of the lymphoid organs that were generally similar between batches and toxicity of the lungs. Although lung findings were minimal to slight the number of incidences were often greater in the TOX1/FTY720 treated M, especially with increased macrophages, collagen and smooth muscle hypertrophy. In F, there were lymphoid and lung findings, but no substantial difference between TOX1/FTY720 and FTY720 treatment.

Summary table of male microscopic findings

		MALE					FEMALE				
Tissue	**Finding**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Pituitary	Rathke's cleft dilatation										
	minimal:	**1/10**	**-**	**2/10**	**-**	**3/10**	**1/10**	**0/1**	**1/10**	**-**	**0/10**
	slight	**0/10**	**-**	**1/10**	**-**	**1/10**	**0/10**	**1/1**	**1/10**	**-**	**1/10**
	moderate:	**0/10**	**-**	**0/10**	**-**	**0/10**	**0/10**	**0/1**	**1/10**	**-**	**0/10**
Eye	Inflammation										
	minimal:	**0/10**	**-**	**0/10**	**-**	**0/10**	**1/10**	**0/1**	**1/10**	**-**	**1/10**
	slight:	**0/10**	**-**	**1/10**	**-**	**0/10**	**1/10**	**0/1**	**1/10**	**-**	**1/10**

Tissue	Finding	MALE 1	2	3	4	5	FEMALE 1	2	3	4	5
Eye	Hemorrhage										
	minimal:	0/10	-	0/10	-	0/10	1/10	0/1	1/10	-	1/10
	slight:	0/10	-	0/10	-	0/10	1/10	0/1	1/10	-	1/10
	moderate:	0/10	-	1/10	-	0/10	1/10	0/1	1/10	-	1/10
Harderian gland	Inflammation										
	minimal:	1/10	-	1/10	-	1/10	0/10	0/1	0/10	0/1	1/10
	slight:	1/10	-	0/10	-	3/10	0/10	0/1	0/10	0/1	1/10
	Acinar dilatation										
	minimal:	2/10	-	2/10	-	2/10	1/10	0/1	4/10	0/1	1/10
	slight:	0/10	-	2/10	-	2/10	1/10	0/1	4/10	0/1	1/10
	Vacuolation										
	minimal:	0/10	-	0/10	-	1/10	0/10	0/1	0/10	0/1	1/10
	slight:	0/10	-	0/10	-	1/10	0/10	0/1	1/10	0/1	2/10
	Atrophy										
	minimal:	0/10	-	0/10	-	0/10	0/10	0/1	0/10	0/1	1/10
	slight:	0/10	-	0/10	-	0/10	0/10	0/1	0/10	1/1	1/10
Heart	Value inflammation										
	minimal:	0/10	-	0/10	-	0/10	0/10	-	1/10	-	0/10
Lungs	Increased lymphoid tissue										
	minimal:	4/10	3/10	0/10	0/10	3/10	0/10	0/10	1/10	0/10	1/10
	slight:	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10
	Inflammation										
	minimal:	1/10	0/10	0/10	1/10	1/10	0/10	0/10	2/10	2/10	0/10
	Congestion										
	minimal:	2/10	1/10	2/10	1/10	1/10	1/10	0/10	0/10	0/10	0/10
	Increased macrophages										
	minimal:	2/10	6/10	2/10	5/10	7/10	0/10	0/10	6/10	3/10	6/10
	slight	0/10	0/10	1/10	1/10	1/10	1/10	4/10	1/10	1/10	0/10
	moderate:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
	Collagen										
	minimal:	0/10	0/10	2/10	0/10	6/10	0/10	0/10	4/10	1/10	1/10
	Smooth muscle hypertrophy										
	minimal:	0/10	0/10	2/10	2/10	9/10	0/10	0/10	5/10	1/10	5/10
	slight:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
	Hyperplasia										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	2/10
	Pleura fibrosis										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
	Edema										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Adrenal gland	Lymphocyte Infiltration										
	minimal:	0/10	-	0/10	-	0/10	0/10	0/1	0/10	-	0/10
	slight:	0/10	-	0/10	-	1/10	0/10	0/1	0/10	-	0/10
Parathyroid gland	Fibrosis										
	minimal:	0/10	-	0/9	-	0/9	0/7	0/1	0/10	-	0/10
	slight:	0/10	-	0/9	-	0/9	0/7	0/1	1/10	-	0/10
Jejunum	Dilatation										
	minimal:	0/10	-	0/10	-	0/10	0/10	0/1	0/10	-	0/10
	slight:	0/10	-	0/10	-	1/10	0/10	0/1	0/10	-	0/10
Cecum	Dilatation										
	minimal:	0/10	-	0/10	-	0/10	0/10	0/1	0/10	-	0/10
	slight:	0/10	-	0/10	-	1/10	0/10	0/1	0/10	-	0/10

Tissue	Finding	MALE 1	MALE 2	MALE 3	MALE 4	MALE 5	FEMALE 1	FEMALE 2	FEMALE 3	FEMALE 4	FEMALE 5
Spleen	White pulp atrophy										
	minimal:	0/10	1/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	0/10	5/10	0/10	0/10	0/10	1/10	1/10	2/10	2/10
	moderate:	0/10	1/10	4/10	0/10	7/10	0/10	0/10	2/10	1/10	4/10
	marked:	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10
	Hematopoiesis										
	minimal:	0/10	0/10	1/10	0/10	0/10	3/10	0/10	1/10	0/10	0/10
	slight:	0/10	0/10	0/10	0/10	0/10	0/10	2/10	1/10	0/10	0/10
Thymus	Atrophy										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	3/10	5/10	2/10	3/10	0/10	0/10	4/10	1/10	4/10
	moderate:	0/10	2/10	1/10	0/10	1/10	0/10	0/10	0/10	1/10	1/10
	Hyperplasia										
	minimal:	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10	0/10	0/10
	slight:	0/10	4/10	4/10	1/10	4/10	0/10	2/10	1/10	3/10	5/10
	moderate:	0/10	5/10	0/10	5/10	4/10	0/10	3/10	2/10	5/10	2/10
	marked:	0/10	0/10	0/10	1/10	0/10	0/10	3/10	1/10	0/10	0/10
	Hemorrhage										
	minimal:	2/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	0/10
	slight:	0/10	0/10	1/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	Hemosiderosis										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
Bronchial Lymph Node	RBC accumulation										
	minimal:	4/9	3/10	2/9	1/10	210	4/10	2/10	2/10	1/10	1/10
	slight:	1/9	4/10	6/9	9/10	5/10	1/10	4/10	4/10	5/10	8/10
	moderate:	0/9	0/10	0/9	0/10	0/10	1/10	0/10	0/10	0/10	1/10
	marked:	0/9	1/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Histiocytosis										
	minimal:	0/9	0/10	0/9	0/10	1/10	0/10	0/10	0/10	1/10	0/10
	slight	0/9	0/10	1/9	0/10	0/10	0/10	2/10	4/10	1/10	0/10
	moderate:	0/9	0/10	0/9	0/10	0/10	0/10	1/10	0/10	0/10	0/10
	Atrophy										
	minimal:	0/9	0/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/9	2/10	3/9	1/10	3/10	0/10	0/10	3/10	2/10	0/10
	moderate	0/9	5/10	3/9	6/10	2/10	0/10	2/10	0/10	0/10	2/10
	marked:	0/9	0/10	0/9	0/10	0/10	0/10	0/10	0/10	1/10	0/10
	Angiectasis										
	minimal:	0/9	0/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight	0/9	3/10	1/9	1/10	0/10	1/10	1/10	3/10	0/10	0/10
	moderate:	0/9	0/10	0/9	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Bronchial Lymph Node	Hemosiderosis										
	minimal:	0/9	1/10	0/9	0/10	1/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/9	0/10	1/9	1/10	0/10	1/10	2/10	0/10	2/10	1/10
	Edema										
	minimal:	0/9	0/10	0/9	0/10	0/10	0/10	0/100	0/10	0/10	0/10
	slight:	0/9	0/10	0/9	0/10	0/10	0/10	/10	0/10	0/10	0/10
	moderate:	0/9	0/10	0/9	0/10	0/10	1/10	0/10	0/10	0/10	1/10
Mandibular Lymph Node	RBC accumulation										
	minimal:	0/10	0/10	1/10	0/10	3/10	1/10	0/10	1/10	1/10	1/10
	slight:	4/10	1/10	2/10	3/10	2/10	1/10	2/10	2/10	1/10	5/10
	moderate:	0/10	1/10	1/10	1/10	0/10	1/10	0/10	0/10	0/10	0/10

Tissue	Finding	MALE 1	MALE 2	MALE 3	MALE 4	MALE 5	FEMALE 1	FEMALE 2	FEMALE 3	FEMALE 4	FEMALE 5
Mandibular Lymph Node	Histiocytosis										
	minimal:	1/10	0/10	1/10	0/10	2/10	1/10	1/10	0/10	0/10	0/10
	slight:	0/10	2/10	3/10	2/10	4/10	0/10	6/10	6/10	4/10	4/10
	moderate:	0/10	1/10	0/10	2/10	1/10	0/10	1/10	1/10	0/10	1/10
	Lymphoid hyperplasia										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	moderate:	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10	0/10
	Atrophy										
	minimal:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	2/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
	moderate:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
	marked:	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Hemosiderosis										
	minimal:	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Mesenteric Lymph Node	Congestion										
	minimal:	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	RBC accumulation										
	minimal:	0/10	2/10	1/10	1/10	2/10	0/10	0/10	0/10	1/10	1/10
	slight:	0/10	0/10	2/10	1/10	1/10	0/10	0/10	0/10	1/10	0/10
	Histiocytosis										
	minimal:	0/10	0/10	2/10	0/10	0/10	0/10	0/10	1/10	0/10	1/10
	slight:	0/10	5/10	5/10	2/10	4/10	0/10	4/10	6/10	4/10	2/10
	moderate:	0/10	2/10	3/10	6/10	3/10	0/10	4/10	1/10	3/10	2/10
	Atrophy										
	minimal:	0/10	0/10	1/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	3/10	4/10	1/10	6/10	0/10	1/10	3/10	1/10	4/10
	moderate:	0/10	4/10	1/10	4/10	1/10	0/10	3/10	2/10	1/10	0/10
	marked:	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Angiectasis										
	minimal:	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	1/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
	Hemosiderosis										
	minimal:	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight:	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
Mediastinal Lymph Node	RBC accumulation										
	minimal:	0/1	-	-	-	0/1	-	-	-	-	-
	slight:	1/1	-	-	-	0/1	-	-	-	-	-
	moderate:	0/1	-	-	-	1/1	-	-	-	-	-
	Histiocytosis										
	minimal:	0/1	-	-	-	0/1	-	-	-	-	-
	slight:	0/1	-	-	-	1/1	-	-	-	-	-
Prostate	Decrease secretion										
	minimal:	0/10	1/10	2/10	2/10	4/10	-	-	-	-	-
	slight:	1/10	0/10	2/10	0/10	4/10	-	-	-	-	-
	moderate:	0/10	0/10	1/10	0/10	0/10	-	-	-	-	-

Groups: 1 - Control, 2 - 1 mg/kg, 3 - 10 mg/kg FTY720, 4 - 1.03 mg/kg and 5 - 10.3 mg/kg TOX1/FTY720.

Toxicokinetics

Blood samples were taken at the end of treatment on Days 21/22 at 0.5, 1, 4, 7 and 24 hours post-dose. Two animals/sex/group were sampled at each time point.

No plasma FTY720 was detected in control samples (LLOQ = 9.45 ng/ml). T_{max} was at 7 hours with C_{max} and plasma exposure increased approximate dose-proportionally in M and F. There was a slight increase in plasma exposure in F compared to M and a slight increase in plasma exposure between TOX1/FTY720 and FTY720 groups, especially at the HD.

Table of toxicokinetic parameters (Sponsor's)

	Dose groups							
	2		3		4		5	
Pk parameters	Male	Female	Male	Female	Male	Female	Male	Female
t_{max}	7	7	7	2	7	7	7	2
C_{max}	38.67	48.13	272.81	436.53	47.55	47.70	354.96	494.79
C_{max}/dose	38.7	48.1	27.3	43.7	47.6	47.7	35.5	49.5
AUC(0-24h)	652	849	5086	7082	813	847	7011	8343
AUC(0-24h)/dose	652	849	509	708	813	847	701	834

AUC is calculated by linear trapezoidal integration.
Units: t [h]. C [ng/mL]. C/dose [(ng/mL)/(mg/kg/day)].
Units: AUC [h·ng/mL]. AUC/dose [(h·ng/mL)/(mg/kg/day)].
Missing C(0) has been replaced for AUC calculation by the concentration measured at 24h.

Group 2 – 1 mg/kg, group 3 – 10 mg/kg, group 4 - 1.03 mg/kg and group 5 - 10.3 mg/kg

Stability and Homogeneity

FTY720 and impurities (b) (4) were chemically stable for at least 11 days at 6°C and 4 hours at room temperature. Samples were taken from solutions at different time points that included; initial, 4 hours stirring at room temperature and 11 Days at 6°C. Stability data showed that FTY720 ranged between 99.5-101% of the target concentration after 4 hours at room temperature and 99.5-103% after 11 days at 6°C. Percentage of target values for (b) (4) were 102-106% and 108-113%, for (b) (4) were 100-105% and 98-105%, for (b) (4) were 99.4-101% and 98.2-105% and for (b) (4) were 100-106% and 98.4-116%. The concentrations of impurities were within expected range (±15%) except for the LD of (b) (4) in Week 4 that was 72%. Homogeneity tests were not performed.

Study title: 4-Week oral (gavage) administration toxicity study in the rat

Study no.:	PCS-r0320066
Study report location:	EDR: 4.2.3.7.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2 December 2003
GLP compliance:	Yes (b) (4) 1999)
QA statement:	Yes (Dated: 16 July 2004)
Drug, lot #, and % purity:	*TOX2/FTY720, Batch № 03/1, 85% with 4.6% NAP (b) (4), 4.5% NAP (b) (4) 4.8% NAP (b) (4) FTY720, Batch № 0324029, 99.6%

Key Study Findings

- Lymphocytes were reduced in all groups.
- Histopathological findings included atrophy of lymphoid organs at 3 and 10 mg/kg/day and foamy histiocytes and inflammatory cell foci in the lungs at 10 mg/kg/day.
- There was no substantial difference between the groups treated with FTY720 with (TOX2) or without degradation products.
- These data suggest that the degradation products (b) (4) at the levels used did not cause any further toxicity.

Methods

Doses:	0, 3*, 10* and 10# mg/kg/day see above table (Drug) for * and #
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	10 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Rat/Crl:WI
Number/Sex/Group	
Main study:	10
Age:	9-10 weeks at start of treatment
Weight	
Males:	251-302.4 g
Females:	176.1-218.5 g

Observations and Results

Mortality

One F at the LD died on Day 4 and was considered to be related to a gavage injury.

Clinical Signs

Observations were performed daily. Physical examinations were performed weekly.

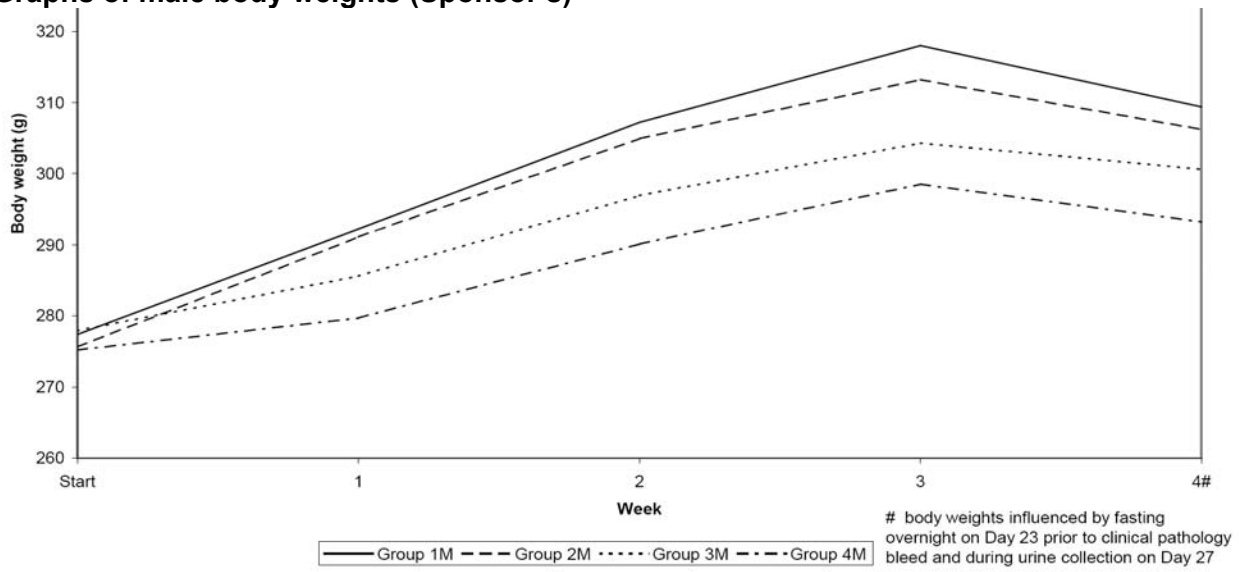
There were no drug-related changes, with the fur thinning and damaged teeth the only observations.

Body Weights

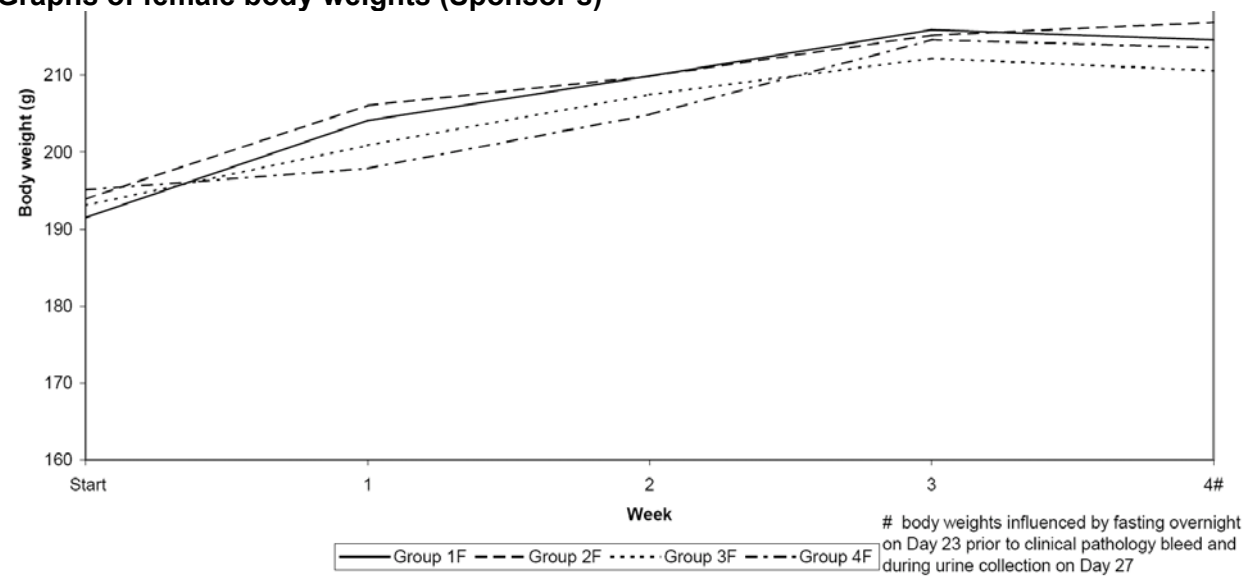
Body weights were recorded once during pre-treatment, on the first day of treatment, weekly during treatment and at necropsy.

There was a decrease in body weight, at Week 3, in M at 10 mg/kg/day TOX2/FTY720 (35%) and 10 mg/kg/day FTY720 (43%) and F at 10 mg/kg/day TOX2/FTY720 (22%) and 10 mg/kg/day FTY720 (20%).

Graphs of male body weights (Sponsor's)



Graphs of female body weights (Sponsor's)



Food Consumption
Food consumption was recorded weekly during treatment.

There was a decrease in food consumption for all treatments, but was greatest over the first week. In M, there was a decrease at 3 mg/kg/day TOX2/FTY720 (3%), 10 mg/kg/day TOX2/FTY720 (10%) and 10 mg/kg/day FTY720 (13%) during Week 1 and a decrease of 2%, 7% and 10%, respectively at the end of the study. In F, there was a decrease at 3 mg/kg/day TOX2/FTY720 (6%), 10 mg/kg/day TOX2/FTY720 (17%) and 10 mg/kg/day FTY720 (19%) during Week 1 and a decrease of 3%, 13% and 12%, respectively at the end of the study.

Ophthalmoscopy
Examinations were performed pre-treatment and in Week 4.

The Sponsor stated that "*No ophthalmological abnormalities were noted*", however no raw data were submitted for review.

Hematology
Blood samples were taken in Week 4. The following parameters, listed in the study report, were assessed.

haemoglobin concentration	red blood cell count
packed cell volume (haematocrit)	reticulocytes
mean cell volume	mean cell haemoglobin
mean cell haemoglobin concentration	haemoglobin distribution width
red cell distribution width	platelet count
platelet crit	mean platelet volume
platelet distribution width	total and differential white cell count

In addition, the following parameters were determined on blood taken into trisodium citrate anticoagulant:

prothrombin time	activated partial thromboplastin time

There was an increase in M and F reticulocytes at all doses. In M, there were decreases in RBC counts and PCV and an increase in red cell distribution width at 10 mg/kg/day TOX2/FTY720 that were statistically different from 10 mg/kg/day FTY720 values.

All treatment groups had a decrease in WBCs. In M and F at 10 mg/kg/day, TOX2/FTY720 and 10 mg/kg/day FTY720, lymphocytes (74-78%) were decreased and neutrophils (40-100%) increased.

Clinical Chemistry
Blood samples were taken in Week 4. The following parameters, listed in the study report, were assessed.

aspartate aminotransferase	alanine aminotransferase
alkaline phosphatase	sodium
potassium	calcium
inorganic phosphorus	chloride
total protein	albumin
globulin	albumin/globulin ratio
total cholesterol	glucose
urea	total bilirubin
creatinine	

There were no remarkable changes.

Urinalysis

Urine samples were taken in Week 4. The following parameters, listed in the study report, were assessed.

microscopy of sediment	volume
specific gravity	pH¤
protein¤	glucose¤
ketones¤	bilirubin¤
blood¤	urobilinogen¤
reducing substances¤	

¤ determined semi-quantitatively.

There was an increase in urine volume in M at 10 mg/kg/day FTY720 and F at 10 mg/kg/day TOX2/FTY720 and a mean decrease in the pH of all treated M. Otherwise, there were no remarkable changes.

Gross Pathology

Gross pathology was assessed in all animals.

The macroscopic findings were generally unremarkable. There was a large number (6) of injection lesions reported in the cecum of F at 10 mg/kg FTY720.

Organ Weights

At necropsy, the organs listed in the table below (from the study report) were weighed.

There was a decrease in the weight of spleen (12-18%) and prostate (24-33%) and an increase in liver (9-12%), lung (19-40%), heart (16-28%) and thyroid (9-15%) weights of all treated M. In M, differences between 10 mg/kg TOX2/FTY720 and FTY720 treatment included increases in lung (28% and 40%, respectively), brain (3% and 7%, respectively), thymus (17% and 3%, respectively) and popliteal lymph node (21% and

32%, respectively). In F, differences between 10 mg/kg TOX2/FTY720 and FTY720 treatment included increases in lung (30% and 40%, respectively), thyroid (10% and -1%, respectively), thymus (9% and 3%, respectively) and decrease in popliteal lymph node (21% and 3%, respectively).

Histopathology

At necropsy the following organs, listed in the study report, were prepared for histopathology examination.

Tissue/organ preserved	Organ weighed	Tissue examined	Tissue/organ preserved	Organ weighed	Tissue examined
adrenals	X	X	nasal cavity (d)		
animal identification			nasopharynx/nares (d)		
aorta		X	oesophagus		X
bone marrow smear (femur) (a) (c)			optic nerves		X
			ovaries	X	X
brain	X	X	pancreas		X
caecum		X	pituitary	X	X
colon		X	prostate	X	X
duodenum		X	rectum		
eyes with optic nerves (b)		X	salivary glands		X
femur with bone marrow and articular surface		X	sciatic nerves		X
			seminal vesicles		X
gross lesions		X	skin		X
Harderian glands (d)			spinal cord (cervical, thoracic, lumbar)		X
head					
heart	X	X	spleen	X	X
ileum		X	sternum with bone marrow		X
jejunum		X	stomach		X
kidneys	X	X	testes with epididymides (e)	X	X
lacrimal glands (d)			thymus	X	X
larynx		X	thyroid (with parathyroid)	X	X
liver	X	X	tongue		X
lung	X	X	trachea		X
lymph node – mandibular		X	trachea bifurcation		
lymph node – mesenteric		X	urinary bladder		X
lymph node – popliteal	X	X	uterus	X	X
mammary area		X	vagina		X
muscle (quadriceps)		X	Zymbal glands (d)		

fixative = 10% neutral buffered formalin except where indicated by:

a – methanol, b - Davidson's fluid, e - Bouin's fixative and processed to block stage
c – see clinical pathology section
d - preserved with the head in situ
Bone designated for histopathological examination was decalcified using a formic acid decalcifier solution as per (b) (4) SOPs.

Adequate Battery: Yes
Peer Review: No, Principal investigator was [redacted] (b) (4)

The findings of foamy histiocytes and inflammatory cell foci in the lungs and nephropathy were observed at 10 mg/kg/day TOX2/FTY720 and FTY720. Other major findings were observed in the thymus, spleen and mesenteric lymph node that included hematopoiesis and atrophy. There was no substantial difference between TOX2/FTY720 and FTY720 treated animals.

Summary Table of microscopic findings

		MALE				FEMALE			
Tissue	**Finding**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Brain	Vacuolation								
	minimal:	**0/10**	**0/10**	**1/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
Pituitary	Cyst								
	present:	**1/10**	**3/10**	**2/10**	**3/10**	**1/10**	**3/9**	**2/10**	**2/10**
Heart	Cardiomyopathy								
	minimal:	**0/10**	**1/10**	**0/10**	**0/10**	**0/10**	**1/9**	**0/10**	**0/10**
Liver	Inflammatory cell foci								
	minimal:	**10/10**	**8/10**	**10/10**	**10/10**	**9/10**	**9/9**	**10/10**	**9/10**
	slight:	**0/10**	**0/10**	**0/10**	**0/10**	**1/10**	**0/9**	**0/10**	**1/10**
	Agonal cong./hemorrhage								
	present:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**1/9**	**0/10**	**0/10**
Lungs	Foamy histiocytes								
	minimal:	**1/10**	**0/10**	**3/10**	**1/10**	**0/10**	**0/9**	**3/10**	**3/10**
	slight:	**0/10**	**0/10**	**1/10**	**1/10**	**0/10**	**0/9**	**0/10**	**0/10**
	Inflammatory cell foci								
	minimal:	**0/10**	**0/10**	**1/10**	**0/10**	**0/10**	**0/9**	**1/10**	**1/10**
	Osseous metaplasia								
	present:	**0/10**	**1/10**	**0/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
Kidneys	Nephropathy								
	minimal:	**1/10**	**2/10**	**3/10**	**4/10**	**0/10**	**1/9**	**4/10**	**2/10**
	slight:	**0/10**	**0/10**	**1/10**	**0/10**	**1/10**	**0/9**	**0/10**	**0/10**
	Inflammatory cell foci								
	minimal:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
	slight:	**0/10**	**0/10**	**1/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
	Pigment								
	minimal:	**0/10**	**0/10**	**1/10**	**0/10**	**3/10**	**3/9**	**1/10**	**2/10**
	Hyaline droplets								
	minimal:	**7/10**	**5/10**	**7/10**	**6/10**	**0/10**	**0/9**	**0/10**	**0/10**
	slight:	**2/10**	**4/10**	**1/10**	**3/10**	**0/10**	**0/9**	**0/10**	**0/10**
	Hydronephrosis								
	minimal:	**1/10**	**2/10**	**1/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
	slight:	**0/10**	**0/10**	**0/10**	**0/10**	**1/10**	**0/9**	**0/10**	**0/10**
	moderate:	**0/10**	**0/10**	**0/10**	**1/10**	**0/10**	**0/9**	**0/10**	**0/10**
	Papillary mineralization								
	minimal:	**1/10**	**1/10**	**1/10**	**0/10**	**0/10**	**0/9**	**1/10**	**0/10**
	Casts								
	minimal:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/9**	**1/10**	**1/10**
Larynx	Adenitis								
	minimal:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/9**	**1/10**	**0/10**

Tissue	Finding	MALE 1	MALE 2	MALE 3	MALE 4	FEMALE 1	FEMALE 2	FEMALE 3	FEMALE 4
Cecum	Barbiturate lysis present:	**0/10**	**0/10**	**1/10**	**0/10**	**1/10**	**0/9**	**0/10**	**5/9**
Thyroid	Ectopic thymus present:	**1/10**	**0/10**	**0/10**	**0/10**	**0/10**	**1/9**	**2/10**	**0/10**
Thymus	Atrophy minimal:	**0/10**	**6/10**	**8/10**	**7/10**	**0/10**	**6/9**	**8/10**	**6/10**
	slight:	**0/10**	**4/10**	**2/10**	**3/10**	**0/10**	**0/9**	**0/10**	**1/10**
	Cyst present:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**1/9**	**1/10**	**1/10**
Thymus	Agonal cong./hemorrhage present:	**0/10**	**1/10**	**1/10**	**0/10**	**1/10**	**0/9**	**0/10**	**0/10**
Popliteal Lymph Node	Agonal cong./hemorrhage present:	**0/10**	**0/10**	**1/10**	**0/10**	**0/10**	**0/9**	**0/10**	**0/10**
	Hyperplasia minimal:	**0/10**	**0/10**	**0/10**	**1/10**	**0/10**	**0/9**	**0/10**	**0/10**
Mesenteric Lymph Node	Atrophy minimal:	**2/10**	**7/10**	**9/10**	**5/10**	**3/10**	**9/9**	**8/10**	**8/10**
	slight:	**0/10**	**2/10**	**1/10**	**5/10**	**0/10**	**0/9**	**2/10**	**2/10**
Spleen	Hematopoiesis minimal:	**2/10**	**3/10**	**5/10**	**4/10**	**2/10**	**3/9**	**3/10**	**5/10**
	slight:	**0/10**	**0/10**	**0/10**	**2/10**	**2/10**	**6/9**	**5/10**	**3/10**
	Atrophy minimal:	**0/10**	**5/10**	**7/10**	**5/10**	**0/10**	**5/9**	**6/10**	**5/10**
	slight:	**0/10**	**0/10**	**1/10**	**4/10**	**0/10**	**1/9**	**3/10**	**3/10**
Adrenal gland	Hemangiectasis minimal:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**1/10**	**0/10**	**0/10**
	Cortical hypertrophy minimal:	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**2/10**

Does not include female LD № 57 died Day 4. Cong. - congestion
Group 1 - control, Group 2 - 3 mg/kg/day TOX2/FTY720, Group 3 - 10 mg/kg/day TOX2/FTY720 and Group 4 - 10 mg/kg/day FTY720.

Toxicokinetics

Blood samples were taken at the end of treatment on Day 28 at 0.5, 1, 4, 8 and 24 hours post-dose.

No plasma FTY720 was detected in control samples (LLOQ = 2 ng/ml). T_{max} was at 4 hours with plasma C_{max} and AUC increasing approximately dose-proportionally in M and F. Generally, there were no gender differences or consistent differences in plasma exposure to TOX2/FTY720 and FTY720.

Table of toxicokinetic parameters

	Group 2		Group 3		Group 4	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
T_{max} (h)	**4**	**4**	**4**	**4**	**4**	**4**
C_{max} (ng/ml)	**102**	**107**	**353**	**322**	**370**	**479**
C_{max}/Dose	**34.0**	**35.7**	**35.3**	**32.2**	**37.0**	**47.9**
AUC_{0-24h} (ng*h/ml)	**1,520**	**1,890**	**7,360**	**6,090**	**6,360**	**8,000**
AUC_{0-24h}/Dose	**507**	**629**	**736**	**609**	**636**	**800**

Stability and Homogeneity
FTY720 was prepared daily and was stable with deviation from the expected concentrations of -0.3% to -1.7%. Homogeneity test demonstrated that solutions were homogeneously distributed; however, the LD TOX2/FTY720 was outside the accepted deviation with values of -20.8% to -25.2% from the expected concentration, the HD TOX2/FTY720 and FTY720 solutions were with in accepted deviation.

10.2 Antigenicity

The Antigenicity of FTY-720 was assessed in mice and guinea pigs. M mice sensitized to FTY720 gave negative passive cutaneous anaphylaxis reactions; there was no anti-FTY720 IgE antibody produced. Therefore, FTY720 had no antigenic potential to induce IgE antibody in mice. In guinea pigs, sensitized with 0.01 and 1 mg/kg FTY720, no skin reactions, passive cutaneous anaphylaxis reactions or systemic anaphylaxis were observed after challenge with FTY720.

10.3 Immunotoxicity

In mice, a single dose of FTY720 inhibited the challenge induced hyperplasia in lymph nodes directly after administration, but did not affect the relative counts of CD4 blastocytes and the expression of the IL-2 receptor on CD4 T-cells or the *in vitro* cytokine release by restimulated CD4 T-cells and antigen presenting cells, suggesting that the immune memory function for contact allergens was not affected. In monkeys FTY720 inhibited tetanus toxoid-specific IgG response in naïve animals. In contrast, tetanus toxoid-IgG response occurred in the presence of FTY720 in primed animals. These data suggest that FTY720 does not interfere with memory T-cells, but does interfere with naïve T-cells.

In naïve mice, lymph node hyperplasia induced by DNCB (induced on Days 2-4) was decreased after multiple doses (4 days) of 0.1 and 1 mg/kg FTY720. However, no change in the level of CD4 T-cell activation was observed. In mice, pre-sensitized to DNCB, multiple doses (7 days) of 0.1 and 1 mg/kg FTY720 challenge by DNCB dose-dependently decreased lymph node hyperplasia. Decrease lymph node weight and increase in macrophage activity seen in FTY720 treated animals was reversed after recovery, showing that the effect of FTY720 was reversible. No effect on CD4 T-cell activation or cytokine expression was observed.

In the 26 Week rat toxicity study, approximately 4 weeks before necropsy, 6 F from each group were treated to assess the sheep red blood cell (SRBC) immune response. The challenge was performed 3 weeks later by i.v. injection of SRBC (same concentration and volume) with blood samples taken at necropsy to assess SRBC agglutination; an indicator of the presence of SRBC-specific IgM antibodies in the serum. All animals generated SRBC specific IgM, but in FTY720 treated animals there was a decrease in SRBC specific IgM, however, it was not dose-dependent. Further, immunohistochemical staining was performed on thymuses of 5 M at control and HD

(7.5 mg/kg), using monoclonal proliferating cell nuclear antigen (PCNA) antibody, rabbit anti human CD3 T-cell antibody and mouse anti rat CD8 antibody. PCNA positive cells were decreased in thymuses at the HD, CD3 positive cells from the medulla in thymuses at the control and HD had a similar expression cells and were mainly PCNA negative and there was a slight reduction of CD8 positive cells in the cortex of thymus at the HD. This suggests, that FTY720 decreases cortical proliferating cells (undifferentiated T-cells), but not CD3 positive cells (differentiated T-cells).

10.3 Lung mechanistic studies

FTY720 did not increase the proliferation of human bronchial smooth muscle cells in culture. In mice and rats, FTY720 (i.v.) caused an immediate increase in MRI signal intensity suggestive of bronchoconstriction. The acute affects of FTY720 on airway resistance was reduced with pretreatment of *B. pertussis* toxin suggesting the involvement of Gi-GTP signaling pathways. Activation of the plasminogen pathway and phagocytic cells were implicated in the acute action of FTY720 as the protein and mRNA of a marker for the activation of plasminogen pathway, PAI-1, and the mRNA of a marker for activation of phagocytic cells, ATF3, were upregulated after FTY720 treatment. In the 52 Week monkey study, labored breathing was observed in most HD animals sedated with ketamine after chronic administration of FTY720. This was also observed in rats, with acute FTY720 alone significantly increasing PENH (enhanced pause), suggesting airway obstruction, with the effect potentiated by ketamine. In addition, smooth muscle hypertrophy at the terminal bronchioles and alveolar ducts were observed.

11 Integrated Summary and Safety Evaluation

FTY720 is a structural analogue of sphingosine that is phosphorylated by sphingosine kinases to the active moiety, FTY720-P. FTY720 has high affinity (0.3-3.1 nM) at sphingosine 1-phosphate (S1P) receptors; $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$, but not $S1P_2$ (>10 μM), causing cells to become insensitive to S1P and preventing the recirculation of cells. FTY720 also had affinity at a number of other human receptors, although at much lower K_i's (1-10 μM), FTY720-P showed no affinity at these targets. Functionally, FTY720 administered daily has been demonstrated to prevent the onset and relapse of EAE signs in F rats.

In safety pharmacology studies, oral administration of FTY720 up to 10 mg/kg had no effect on alertness, locomotor activity and other behaviors in mice, but decreased rotorod performance from 3 mg/kg; phenobarbital-induced sleep was prolonged in rats. The hERG tail current was inhibited to a similar level by FTY720 (0.5 μM) and FTY720-P (0.5 μM), 25% and 18%, respectively, with blood pressure increased in dogs after 1 mg/kg. Heart rate was decreased in monkeys administered 1 and 10 mg/kg and blood pressure increased. There were no changes in respiratory rate, oxygen saturation, arterial oxygen and carbon dioxide tension. Changes in renal function suggested a decrease in glomerular filtration rate and an increase in sodium reabsorption.

Oral bioavailability of FTY720 was high in all species, with bioavailability in human the highest (estimated at 93%). However, because of the inter-conversion of FTY720 with FTY720-P, an accurate estimate of oral bioavailability is not possible. Absorption of FTY720 in animals after oral administration was slow, but was well absorbed in all species; FTY720 and FTY720-P disappeared from blood approximately in parallel. Plasma protein binding for FTY720 was high (close to 100%), whereas FTY720-P binding was more variable. Tissue distribution was extensive, with steady state volume of distributions of 17.3, 21 and 7.6 l/kg for mice, rats and dogs, respectively. Using modeling data, rat and dog steady state brain concentrations were estimated to be achieved after 23-36 days; 7.9-14.7-fold accumulation is expected.

The biotransformation of FTY720 in humans occurs by 3 main pathways; reversible stereoselective phosphorylation, oxidative biotransformation mainly via the cytochrome P450 4F2 isoenzyme and subsequent fatty acid-like degradation and formation of nonpolar ceramide analogs. The main FTY720-related components in human blood plasma are FTY720, FTY720-P and metabolites M2 (1.5%), M3 (8.3%), M29 (8.9%) and M30 (7.3%). *In vivo,* FTY720-P and FTY720 were the main components in mouse plasma. In rat, M3 and M23 were primary metabolites, with M2, M4, M5, M14, M20, M24, M27, M27, M29 and M30 also detected. In dog, levels of M3 were also high and other metabolites included M1, M2, M4 and M30. In monkey, M3 was also the main metabolite with other metabolites including M2, M7, M29 and M30. Main metabolites excreted via the urine were M3 and M5, whereas FTY720 and FTY720-P were not

found in urine. Fecal excretion included the main metabolites found in rat, M1 and M2, and in dog, M1, M2, M3 and FTY720-P.

The primary human metabolites, M2 and M3 were assessed in the 26-Week rat and 39-Week monkey toxicity studies, with plasma exposure (AUC_{0-24}) measured on Day 1-2 for rat of 61.5 and 1240 ng*h/ml, respectively and for monkey of 256 and 567 ng*h/ml, respectively. Human plasma levels for these 2 metabolites after a single oral dose (5 mg) were 43 and 218 ng*h/ml, respectively. Therefore, extrapolating these human levels of metabolites after 5 mg administration for a 0.5 mg dose ($^{1}/_{10}^{th}$) would suggest plasma exposure, after a single dose, of both metabolites less than levels measured in both species. In addition, steady-state human plasma exposure (AUC_{ss}) after daily repeated treatment of 0.5 mg FTY720 was 73 ng*h/ml after 28 Days in renal transplantation patients (pharmacokinetics are considered similar to healthy and multiple sclerosis patients), allowing estimates of M2 and M3 plasma exposure of 4.7 and 24.0 ng*h/ml, respectively. These plasma exposures are also less than that found in rat and monkey. However, as exposure to these 2 metabolites can be increased in individuals with certain other complications, such as renal problems, metabolite exposure may require further study.

Studies demonstrated that pharmacokinetic parameters of FTY720, when combined with cyclosporine A, 42-O-(2-hydroxyethyl)-rapamycin, sodium mycophenolate or methotrexate, were unaffected.

In single dose studies with FTY720 in mice, rats and dogs, death occurred at 50 mg/kg in mice and M rats and at 25 mg/kg in F rats. In contrast, FTY720 was tolerated up to 2000 mg/kg in dogs. The main target organ in the single dose studies was the lung.

In repeat dose toxicology studies, FTY720 was administered to several different species for 2-52 weeks. FTY720 was lethal in rats and dogs; there were deaths in rats after repeated dose at 30 mg/kg for 5-6 days; in dog, deaths occurred with administration at 10 mg/kg after at least 7 weeks. Few clinical signs were observed with treatment of FTY720; however, breathing difficulties were observed in dogs at 10 mg/kg and monkeys at $\geq$3 mg/kg, especially while under sedation (ketamine). Decreased body weight occurred with treatment with FTY720; in rats at doses greater than 0.5 mg/kg, in dogs at doses greater than 10 mg/kg and in monkeys at doses greater than 1 mg/kg. WBCs and lymphocytes were greatly reduced in all species at all doses, with accompanying histopathological changes of atrophy in the lymphatic organs (spleen, thymus and lymph nodes) in all species. Theses changes, although adverse, can be attributed to the pharmacological, immunosuppressive, action of the drug. There was a decrease in lymphocytes in lymphoid organs reported in rats and dogs, in an apparent contradiction to the mode of action, i.e., prevention of egress of lymphocytes from lymphoid organs. However, in the rat toxicity studies, although there was a decrease in lymphoid area, the density of T-cells was increased in the thymus, but not the spleen.

The main target organs for FTY720 toxicity were heart and lungs. Heart findings observed in mice, rats, dogs and monkeys included heart weight increase and

microscopic changes of fibrosis, necrosis and arterial wall thickening. The dog was the most sensitive species and also included effects on the papillary muscle. NOAELs for heart toxicity in chronic and subchronic studies were 5 mg/kg for mice (13 weeks), 1.5 mg/kg for rats (26 weeks), 0.01 mg/kg for dogs (26 week) and 0.5 mg/kg for monkeys (39 weeks). These convert to 15, 9, 0.2 and 6 mg/m^2, respectively. With a proposed human dose of 0.3 mg/m^2, there is no safety margin for heart toxicity observed in dogs.

Findings of lung toxicity were observed in mice, rats, dogs and monkeys. Lung weight was increased and the most consistent microscopic findings in all species were smooth muscle hypertrophy and macrophage infiltration/accumulation. Other microscopic findings in the lungs included increased collagen and fibrin in rats, dogs and monkeys that result in the lungs becoming more rigid as demonstrated macroscopically with lungs not collapsing at necropsy. Furthermore, the usually uncommon finding of bone formation, osseous metaplasia, was observed in rodents. These changes were generally observed in small terminal bronchioles, alveolar ducts and the entrance to alveolar sacs. In addition, observations of accumulation of macrophages, may explain a number of the deaths that had foamy contents in the respiration system, although as mentioned by the Sponsor, this can also result from mis-dosing. The lung changes may also explain the apparent reduced respiratory function observed in monkeys (52 Weeks), especially while under sedation. Other clinical signs, associated with effects on lungs, were dyspnea and rales. NOAELs for lung toxicity were 0.5 mg/kg for mice (13 Weeks), 0.03 mg/kg for rats (26 Weeks), 0.01 mg/kg for dogs (26 Weeks) and less than 0.5 mg/kg for monkeys (39 Weeks; no NOAEL was established) and had a tendency to decrease as the duration of study increased. These NOAELs convert to 1.5, 0.18, 0.2 and <6 mg/m^2, respectively, and makes the rat and dog the most sensitive species for lung toxicity, although the monkey level is uncertain due to the lack of an NOAEL. The human dose on a body surface area basis is 0.3 mg/m^2, leaving no margin of safety in at least 2 species.

Findings in other organs included basophilic tubules in rat kidney, decreased secretory production in rat prostate, vacuolation in rat and dog adrenal glands and dog pituitary gland. There was mononuclear infiltration observed in dog brain, degeneration of dog peripheral nerves and monkey nerve fibers. At high doses (10 and 30 mg/kg/day), dogs had findings of perivascular monocyte infiltration of the brain (gray matter), peripheral nerve degeneration (axon and Schwann cells) of the heart, ganglion cell vacuolation of Auerbach's plexus (stomach).

The 104 Week mouse carcinogenicity study was positive for malignant lymphoma, with an increased incidence of malignant lymphoma observed in M and F at the MD and HD. However, the study appears to be inadequate as there were high levels of autolysis and data were frequently miscoded in the dataset. In the rat carcinogenicity study, treatment of F at the HD was terminated due to increased mortality in Week 75 and the entire group was terminated at Week 95, with the second control group; the study was not compromised since there were 4 dose groups. The study was negative with no statistically significant increase in tumor findings.

In genetic toxicity studies, FTY720 did not demonstrate mutagenic potential in the Ames assay and mouse lymphoma tk assay, clastogenic potential in CHL cells and V79 Chinese hamster cells or clastogenic potential in the *in vivo* mouse micronucleus assay. However, the lack of FTY720-P, the active moiety, observed in *in vitro* rat hepatocytes would bring into question the validity of the S9 data.

Summary table of fetal incidences from reproduction and developmental studies

Species	Dose (mg/kg)	Cesarean Data	Viable Fetus	Visceral		Skeletal		NOAEL (mg/kg)
				Malformations	Variations	Malformations	Variations	
Rat Fertility & early embryo	0 1 3 10	Increased Pre-Implant loss at LD and HD						< 1
Rat Embryo fetal develop't	0 1 3 10	Increased Post-Implant loss at all doses	Decreased at all doses (0 at HD)	16/140 (11.4%) 69/117 (59.0%) 4/8 (50%) NP	0/140 (0.0%) 0/117 (0.0%) 0/8 (0.0%) NP	1/152 (0.7%) 2/126 (1.6%) 0/11 (0.0%) NP	61/152 (40.1%) 59/126 (46.8%) 9/11 (81.8%) NP	< 1
Rat Embryo fetal develop't	0 0.03 0.1 0.3	No changes	No change	13/146 (8.9%) 14/141 (9.9%) 31/139 (22.3%) 62/136 (45.6%)	None	0/152 (0.0%) NE NE 0/147 (0.0%)	38/152 (25.0%) NE NE 50/147 (34.0%)	0.03
Rabbit Embryo fetal develop't	0 0.01 0.03 0.3 3	Increased Resorption	No change	4/23 (17.4%) NE NE 8/24 (33.3%) 14/29 (48.3%)	None	0/25 (0.0%) NE NE NE 1/32 (3.1%)	7/25 (28.0%) NE NE NE 13/32 (40.6%)	ND
Rabbit Embryo fetal develop't	0 10 30 60	Abortions all doses	Decreased at all doses	NP	NP	NP	NP	ND
Rabbit Embryo fetal develop't	0 0.5 1.5 5	Increased Resorption	Decreased at MD, HD	0/158 (0.0%) 0/160 (0.0%) 2/159 (1.3%) 1/92 (1.1%)	0/158 (0.0%) 1/160 (0.6%) 2/159 (1.3%) 4/92 (4.3%)	0/158 (0.0%) 1/160 (0.6%) 1/159 (0.6%) 5/92 (5.4%)	135/158 (85.4%) 135/160 (84.3%) 142/159 (89.3%) 89/92 (96.7%)	0.5

NE – not examined, ND – not determined, NP – not performed

In reproductive and developmental studies, there were problems with several of the studies that include: insufficient pregnant dams (<16) at 3 mg/kg/day (MD) examined in the fertility and early embryonic development study, lack of examination of fetuses at 0.03 (LD) and 0.1 mg/kg /day (MD) for skeletal findings in the low dose rat embryo-fetal development study and lack of examination of fetuses at 0.01 (LD) and 0.03 mg/kg/day (MLD) for visceral findings and 0.01 (LD), 0.03 (MLD) and 0.3 mg/kg/day (MHD) fetuses for skeletal findings in the low dose rabbit embryo-fetal development study.

Fertility was not affected in rats up to 10 mg/kg/day. In rat embryo-fetal development studies, FTY720 was fetal lethal and teratogenic for heart-vessel and skeletal systems. A fetal NOAEL was not established in the first study; therefore, it was less than 1 mg/kg/day. A second, low dose study was invalid, as the LD (0.03 mg/kg/day) and MD (0.01 mg/kg/day) were not analyzed for skeletal findings. The Sponsor states that the NOAEL for fetuses was 0.03 mg/kg/day. In rabbits, although 2 studies were invalid, due to the lack of examination of all fetuses, a third embryo-fetal development study established the NOAEL for fetuses at 0.5 mg/kg/day, as there was a significant increase

in embryo-fetal mortality at 1.5 and 5 mg/kg/day. There were also observations of visceral and skeletal malformations in the 1.5 and 5 mg/kg/day fetuses, however these did not reach the level of concern for tetratogenicity. Therefore, FTY720 is teratogenic in the rat causing heart-vessel, such as increased persistent truncus arteriosus, ventricular septal defect and skeletal changes, such as increased incidence of bilateral twin thoracic bodies. A NOAEL for rat was not established, the NOAEL in rabbit was 0.5 mg/kg/day with a plasma exposure of 16 ng*h/ml, this is only 4.6-fold the proposed human dose (AUC_{ss} for 0.5 mg (28 days) was 73 ng*h/ml).

FTY720 and metabolites were measured in rat milk up to 48 hours post-dose and suggests that nursing pups would be exposed to FTY720. Furthermore, placental transfer of FTY720 and metabolites occurred in rats and rabbits.

There were 10 impurities and degradation products identified as potentially genotoxic. Some of the studies submitted to address the genetic toxicity potential of these were not adequate. Although the levels of impurities are below set levels, the genotoxic battery of tests for (b) (4) are invalid and therefore are unacceptable if values are not kept below the recommended levels. Two 4 week toxicity studies were submitted to investigate impurities (b) (4) and degradation products (b) (4); however, 4 week studies are not long enough and should be at least 3 months in duration. The groups treated with TOX1/FTY720 appeared to exhibit a slight increase in the severity of toxic effects. These data suggest that the impurities (b) (4) at the levels used slightly exacerbated the toxicities observed with FTY720. In the FTY720/TOX2 study, there was no substantial difference between the groups treated with or without degradation products suggesting that the degradation products (b) (4) at the levels used did not cause any further toxicity. These 4-week studies are not adequate to qualify the impurities and degradants above the NMT specification level ((b) (4) respectively).

Table of organ NOAELs.

Toxicity	Species	Study	NOAEL (mg/kg)	Safety Margin Based on AUC*
Lungs	Mouse	13 week	0.5	8.9
	Rat	26 week	0.03	No AUCs calculated
	Dog	26 week	0.01	No AUCs calculated
	Monkey	39 week	<0.5	<5.5
Heart	Mouse	13 week	>5	>92.9
	Rat	26 week	1.5	31.4
	Dog	26 week	0.01	No AUCs calculated
	Monkey	39 week (HR&QTc)	<0.5	<5.5
Brain and spinal cord	Mouse	13 week	>5	>92.9
	Rat	26 week	1.5	31.4
	Dog	26 week	1	No AUCs calculated
	Monkey	52 week	>10	No AUCs calculated
Peripheral nerve	Dog	26 week	1	No AUCs calculated
	Monkey	52 week	3	No AUCs calculated
Embryo-fetal	Rat		<1[#]	No TK performed
	Rabbit		0.5	4.6
Pre- and postnatal	Rat		<0.05	No TK performed

*AUC_{ss} for 0.5 mg (28 days) was 73 ng*h/ml (study №: FTY720b102-pk), # studies inadequate.

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/s/

RICHARD J SIAREY
09/20/2010

LOIS M FREED
09/20/2010
Please see memo for comments.

Executive CAC
Date of Meeting: 11 May 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Hanan Ghantous, Ph.D., DAVP, Alternate Member
Lois Freed, Ph.D., DNP, Supervisor
Richard Siarey, Ph.D., DNP, Presenting Reviewer

Author of Minutes: Richard Siarey

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #: 22-527
Drug Name: Fingolimod (FTY720)
Sponsor: Novartis

Background
The Sponsor submitted the final reports for the 2-year mouse and rat carcinogenicity studies on 17 March 2005 to IND 57,293 (DAIDP; withdrawn 19 Feb 2008). At that time the review of the studies "*…identified inconsistencies (e.g., the number of tumors throughout the submission, the number of animals with autolytic tissues, and inconsistent diagnoses throughout the submission), which cannot be resolved with the individual line listings provided, and are not specific to any tissue*". This information was conveyed to the Sponsor via a teleconference on 12 May 2005 (see Memorandum of telecon: IND 57,293, 29 Nov 2005) at which time the Division requested that the histological slides be re-examined. On 10 Oct 2005, the Sponsor proposed a revised plan for re-examination of tissues for both carcinogenicity studies. The Division responded (16 Nov 2005) as follows:

- ***The FDA agreed that "all previously reported tumors and preneoplastic/hyperplastic lesions from all animals should be reviewed, and all the target tissues in all the animals in the mouse and rat final carcinogenicity study reports (001039 and 001040, respectively) should be reviewed".***
- ***The FDA disagreed that" a review of 10% of all tissues in all animals is adequate. As we stated in our facsimile correspondence dated July 13, 2005, at least 20% of all tissues of all animals from both studies should be reviewed".***
- *The following target tissues should be examined for non-neoplastic lesions:*
    - *Mouse Carcinogenicity study 00103: lymphatic organs (thymus, spleen, axillary and mesenteric lymph nodes), lung, kidneys, bone marrow, ocular, and liver.*
    - *Rat Carcinogenicity study 001040: lymphatic organs (thymus, spleen, axillary and mesenteric lymph nodes), kidneys, vascular system (to include mesenteric/pancreatic vessels and heart), brain, lung, and liver.*

Amended study reports for the mouse and rat carcinogenicity studies were submitted in the original NDA; electronic datasets were not included, but were submitted to the NDA on 20 Jan 2010 (SDN 0005). For both studies the peer review of the histopathological findings was performed by an external pathologist (b) (4) between 4 Sep 2006 and 25 Jan 2007 for the mouse study and 20 Feb 2006 and 24 Jan 2007 for the rat study, in a manner that was consistent with the prior agreement with the Agency.

Mouse Carcinogenicity Study

FTY720 was administered to CD-1 mice at doses of 0 (water), 0 (water), 0.025, 0.25, and 2.5 mg/kg/day by oral gavage for up to 104 Weeks. Dosing was stopped in HD males during Week 92 due to decreased survival rate. The survival rate was also decreased in HD females, but the effect was not statistically significant. All dose groups consisted of >10 animals/group at termination of the study. There was a high incidence of autolysis in females (13/60, 12/60, 9/60, 14/60, and 5/60, respectively) and of either severe or partial autolysis in males (15-28%, with no differences among groups). In addition, data appear to have been frequently miscoded in the electronic dataset, such that it was not possible to fully determine which organs were truly not examined and which were miscoded.

There was a statistically significant increase in the incidence of malignant lymphomas in MD and HD females and males: 1/60, 8/60, 5/60 10/60 and 18/60 for males and 10/60, 13/60, 12/60, 25/60 and 24/60 for females at doses of 0, 0, 0.25, 0.25 and 2.5 mg/kg/day, respectively.

Rat Carcinogenicity Study

FTY720 was administered to Wistar rats at doses of 0 (water), 0 (water), 0.05, 0.15, 0.5, and 2.5 mg/kg/day by oral gavage for up to 104 Weeks. There was increased mortality in HD animals, with 29.4% of males and 62.8% of females dead by Week 78. Dosing of HD females was stopped in Week 75 and the entire group was terminated (along with one control group) in Week 95. Dosing of HD males was stopped in Week 99, but survivors were continued to scheduled termination.

No drug-related tumors were detected in either males or females.

Executive CAC Recommendations and Conclusions:

Mouse:

1. The Committee considered the adequacy of the mouse carcinogenicity study to be uncertain, noting the high incidence of autolysis (particularly in females), and recommended that the division:

- ask the Sponsor to address the high incidence of autolysis and the frequent miscoding of data in the electronic dataset, and

- consider any other appropriate actions.

2. The Committee concluded that the malignant lymphomas were drug related in both mid and high dose males and females.

Rat:

The Committee concluded that the rat carcinogenicity study was adequate and that there were no drug-related neoplasms.

David Jacobson Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DNP
/LMFreed, DNP
/RSiarey, DNP
/HToure, DNP
/ASeifried, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22527	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	FINGOLIMOD HCL ORAL CAPSULES

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/s/

ADELE S SEIFRIED
05/20/2010
Signing for David Jacobson Kram