- **Toxicokinetics** A summary of the toxicokinetic (mean) parameters is provided in the following table.

Tacrolimus Concentration (% w/w)	Sex	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{0-24\ hr}$ (ng·hr/ml)	
		Day 1	Day 29	Day 1	Day 29	Day 1	Day 29
0.1	Male	43.7	12.5	6	12	61.6	150.2
	Female	53.4	15.3	2	6	88.0	120.9
0.3	Male	19.7	17.1	2	6	95.2	175.4
	Female	38.3	15.6	2	8	97.5	167.3
1.0 (Intact Skin)	Male	31.2	70.2	2	4	269.5	923.2
	Female	49.8	77.1	12	4	536.9	920.4
1.0 (Abraded Skin)	Male	57.6	70.7	4	2	319.7	623.0
	Female	82.1	73.2	2	2	282.3	668.0

FR900506 was absorbed into systemic circulation following topical ointment administration to rats. The peak concentration in plasma was reached between 2 – 12 hours post dose. $AUC_{0-24\ hr}$ levels increased in a dose dependent manner. AUC values increased 2-3 fold from Day 1 to Day 29 indicating a potential for drug accumulation after repeat dose administration. No apparent sex difference in systemic absorption was noted in this study. In general, the abraded skin and intact skin 1% test gel groups had comparable levels of systemic exposure.

Key Study Findings:

Potential target organs consisting of kidney, lymph nodes (cervical and mesenteric), spleen, thymus and pancreas were identified in this study. Target organs identified in this study are consistent with the pharmacologic effect (immunosuppressant) of tacrolimus. Treatment related findings were observed in both high dose groups and to a lesser extent in mid dose group animals. Skin related effects noted at the treatment site were mainly attributed to placebo gel. No significant differences were noted in the toxicity profile after administration of the high dose to intact vs abraded skin. This result indicates that FR900506 passes readily through intact rodent skin from the FR900506 ointment formulation (confirmed by toxicokinetic data). Minor toxicologic effects noted for the low dose group were probably related to the pharmacological action of FR900506 (immunosuppresant). Therefore, the NOAEL for this study was 0.1% FR900506 ointment (2 mg/kg/day; 12 mg/m^2/day; Day 29 $AUC_{0-24\ hr}$ = 135.5 ng·hr/ml) in the rat.

Repeat Dose Toxicology Study #3:

Toxicity to rats by repeated dermal administration for 26 weeks

Study Title: Toxicity to rats by repeated dermal administration for 26 weeks
Study No: R96-0107-506-P2-E
Amendment #, Vol #: 000, 12-14
Conducting laboratory: [redacted]

Date of study initiation: 5/25/95
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

Approximately 24 hours before treatment commenced, hair was clipped from the dorsal region of each animal. Hair clipping was repeated on an as needed basis. The treatment area was 5 cm x 5 cm (10% of total body surface). All treatment sites were intact skin and unoccluded. Each dose (2 gm/kg/day) was applied to the appropriate dermal test site as a thin uniform layer. The animals were then fitted with a plastic Elizabethan collar for a six hour period. At the end of this period, the collar was removed and the dermal application site was washed with warm water and blotted dry. The treatment site remained unoccluded through out the treatment period. Animals were treated once daily for 26 weeks.

Dosing:

- *species/strain:* Crl:CD Br Sprague-Dawley rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 10 weeks
- *weight:* 304-387 grams males; 217-277 grams females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Study Animals	
		Males	Females	Males	Females
Sham Control	0	20	20	--	--
Placebo Control	0	20	20	--	--
0.03% FR900506 ointment	0.6	20	20	20	20
0.1% FR900506 ointment	2	20	20	20	20
0.3% FR900506 ointment	6	20	20	20	20
0.5% FR900506 ointment	10	20	20	20	20

Note: The dose range selected for this study was based on the results of the 28 day dermal toxicity study conducted in rats described previously. The sponsor determined that the 1.0% FR900506 ointment concentration exhibited a high toxicity and would not be tolerated in a study for 26 weeks duration. This was an acceptable dose range for the 26 week rat dermal toxicity study.

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 702254K
0.03% FR900506 ointment – lot#702354K
0.1% FR900506 ointment – lot# 702454K

0.3% FR900506 ointment – lot# 702554K and 707557K
0.5% FR900506 ointment – lot# 702654K and 702754K

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* daily prior to next dose administration for the first 4 weeks and then on a weekly basis
- *Body weights:* weekly
- *Food consumption:* weekly
- *Ophthalmoscopy:* prior to treatment, during weeks 13 and 26
- *Hematology:* during weeks 13 and 26
- *Clinical chemistry:* during weeks 13 and 26
- *Urinalysis:* during week 13 and 26
- *Gross pathology:* at sacrifice
- *Organs weighed:* adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes (with epididymides), thymus (where present), thyroid and uterus
- *Histopathology:* The following organs were preserved in 10% buffered formalin: adrenals, aorta, brain (medullary, cerebellar and cerebral sections), caecum, colon, duodenum, eyes, esophagus, femur, harderian glands, head, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (cervical and mesenteric), mammary glands, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin (treated and untreated), spinal cord (cervical level), spleen, sternum, stomach, testes (with epididymides), thymus, thyroid (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix), vagina and any macroscopically abnormal tissue.

  All tissues and organs were examined for the sham control, placebo control and 0.5% FR900506 treatment groups. The brain, femur, gastro-intestinal tract, kidneys, liver lungs, pancreas, salivary gland, seminal vesicles, skin (treated and untreated), spinal cord, spleen, thymus and lymph nodes (cervical and mesenteric) were examined for the 0.03%, 0.1%, and 0.3% FR900506 intact skin treatment groups.

- *Toxicokinetics:* Blood samples were obtained from toxicokinetic animals on day 1 and weeks 13 and 26. Blood samples taken at 6 and 24 hours after dose application only for each timepoint (10 rats/timepoint).

Results:

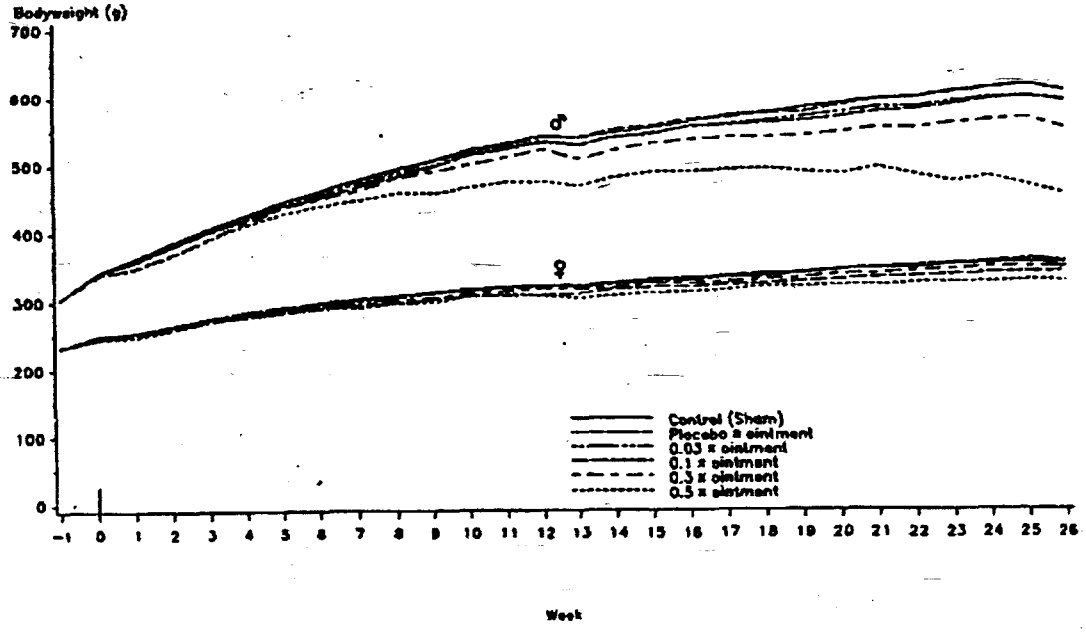
- **Clinical signs** Treatment related deaths were observed for 7 male and 5 female rats treated with 0.5% FR900506 ointment and 5 male and 3 female rats receiving 0.3% FR900506 ointment. These deaths occurred between weeks 9 and 26. The majority of these deaths were preceded by the observation of body tremors (first noted during week 9) and clinical signs indicative of poor health. The clinical signs included unsteady gait, lethargy, labored respiration, leaning to one side, thin appearance, hunched posture, pallor, piloerection, poor grooming and collapse.

- **Local dermal signs** No treatment related dermal effects were noted in this study. However, the contract lab stated that white patch areas or brown staining of the dosing site were noted from Week 2 of treatment in half the animals undergoing treatment with placebo or FR900506 ointment. Occasional animals in the Sham control group also exhibited white patchy areas or brown staining within the shaved area. In all but five instances findings of white patchy areas were confined to males and brown staining of the skin confined to female animals. The contract lab explained these results as probably procedural in nature. It is unclear the reason for the observed finding since this has not been noted in other studies to date.

- **Body weights** A treatment related decrease in bodyweight gain was observed for rats of both sexes receiving 0.5% FR900506 ointment and males receiving 0.3% FR900506 ointment. This effect was the most marked for high dose (0.5%) males during the later half of the study. Effects on body weight are represented graphically in the figure below copied from the electronic NDA review.

FIGURE 1

Bodyweight - group mean values



- **Food Consumption** A progressive increase in food consumption relative to control was observed throughout the study for males treated with 0.5% or 0.3% FR900506 ointment. Slightly higher values for food consumption were noted from week 2 to 16 for females treated with 0.5% or 0.3% FR900506 ointment but the food consumption level was comparable to control levels from week 16 to study termination in female animals in these dose groups.

- **Ophthalmoscopy** A treatment related incidence of lens findings (anterior or posterior capsular opacities, equatorial opacities or anterior sutures) was observed in 0.3% FR900506 ointment treated males and 0.5% FR900506 ointment treated males and females. A higher incidence of corneal opacities was observed in 0.3% FR900506 ointment treated males and 0.5% FR900506 ointment treated males and females. The contract lab stated that the significance of this is uncertain since there is a low backgrcund incidence of the corneal opacities in other groups. However, I would still consider the effects on the cornea significant since there was a higher incidence of these in the higher dose groups. In addition, cataracts were seen in 13 week gavage studies in male rats with as low a dose as 0.3 mg/kg FK506.

- **Hematology** A treatment related decrease in lymphocyte counts and platelet counts compared to placebo control was observed at week 13 (lymphocyte:

↓40%; platelet: ↓14%) and 26 (lymphocyte: ↓48%; platelet: ↓14%) for males receiving 0.5% FR900506 ointment. In addition, a significant decrease in eosinophils (↓56%), basophiles (↓50%), monocytes (↓55%) and large unstained cell counts (↓54%) was noted for males receiving 0.5% FR900506 ointment after 26 weeks of treatment. A treatment related slight increase in packed cell volume, red blood cell count and hemoglobin levels in comparison with placebo control was observed for males treated with 0.5% and 0.3% FR900506 ointment at week 26.

- **Clinical chemistry** Blood urea nitrogen levels were elevated in males in the 0.3% and 0.5% FR900506 ointment groups at both weeks 13 (0.3%: ↑14%; 0.5%: ↑57%) and 26 (0.3%: ↑50%; 0.5%: ↑135%). Blood urea nitrogen levels were elevated in 0.5% FR900506 ointment treated females at weeks 13 (↑10%) and 26 (↑43%). Elevated alkaline phosphatase levels were noted for males in the 0.3% and 0.5% FR900506 ointment groups at week 26 (0.3%: ↑41%; 0.5%: ↑98%). Higher triglyceride levels were observed at both week 13 (0.3%: ↑32%; 0.5%: ↑61%) and 26 (0.3%: ↑56%; 0.5%: ↑60%) for female rats receiving 0.5% and 0.3% FR900506 ointment. Lower glucose levels were observed at week 13 only for female rats in the 0.5% FR900506 ointment group (↓14%).

- **Urinalysis** Urinary volume was increased at week 13 for 0.5% FR900506 ointment treated male and female rats (males: ↑43%; females: ↑33%) and at 26 weeks for male and female rats in the 0.3% and 0.5% FR900506 group (0.3% males: ↑106%; 0.3% females: ↑43%; 0.5% males: ↑151%; 0.5% females: ↑33%). Urinary chloride levels were elevated for male and female rats treated with 0.5% FR900506 ointment and females receiving 0.3% FR900506 ointment at week 13 (0.3% females: ↑103%; 0.5% males: ↑60%; 0.5% females: ↑87%). A similar effect was observed at week 26 for these dose groups and for males receiving 0.3% FR900506 ointment (0.3% males: ↑123%; 0.3% females: ↑233%; 0.5% males: ↑286%; 0.5% females: ↑222%). In addition, lower potassium values were observed for male rats receiving 0.5% FR900506 ointment at week 26 (↓20%). The presence of total reducing substances and glucose was found in the urine of male rats in the 0.5% FR900506 ointment group at week 13. This effect was also detected at week 26 in the urine of males receiving 0.5% and 0.3% FR900506 ointment and in two females treated with 0.5% FR900506 ointment.

- **Organ weights** Treatment related effects on organ weights were noted in the study. Kidney weights were higher than placebo control for male and female rats treated with 0.5% FR900506 ointment (males: ↑36%; females:

↑14%) and seminal vesicle weights were lower than control for male rats in the 0.3% and 0.5% FR900506 ointment groups (0.3%: ↓20%; 0.5%; ↓47%).

- **Gross pathology**

The gross necropsy examination revealed some treatment related effects associated with FR900506 ointment. Kidneys were enlarged for 3/15 male rats and 5/14 male rats treated with 0.3% and 0.5% FR900506 ointment, respectively. A reduction in thymus size was seen in 3/15 male rats and 11/14 male rats treated with 0.3% and 0.5% FR900506 ointment, respectively. A reduction in adipose tissue was observed for the majority of rats of both sexes in the 0.3% and 0.5% FR900506 ointment groups. Distension of gastro-intestinal tissues (stomach, jejunum, ileum, caecum, colon) was observed for a proportion of animals receiving 0.3% and 0.5% FR900506 ointment. Brown staining of the treated skin sites was observed in all treated groups (mainly male rats). An increased incidence of badly groomed fur was noted in all treated groups and the placebo group. This was not observed in sham control animals. Brown staining of the fur (primarily of the cranial and dorsal cervical regions) was noted in many animals treated with FR900506 or placebo ointment but not in sham control rats.

- **Histopathology**

Treatment related changes were noted in the skin. An increased incidence of epithelial hyperplasia/acanthosis was detected in male rats at all dose levels. The lesion did not exhibit any dose relationship at 0.03% to 0.3% and was least pronounced at the 0.5% dose level (0.03%: 16/20; 0.1%: 17/20; 0.3%: 15/20; 0.5%: 9/20). Basal cell degeneration with vacuolation/nuclear debris was noted in some male rats in the 0.1% (4/20) and the 0.3% FR900506 ointment groups (6/20). Female rats exhibited these changes at low incidences in groups treated with placebo or FR900506 ointment.

Treatment related effects were noted in the thymus. Reduced cellularity of the medulla was detected in both sexes treated with 0.3% and 0.5% FR900506 ointment (0.3% males: 20/20; 0.3% females: 19/20; 0.5% males: 20/20; 0.5% females: 20/20). An increased incidence of cortical apoptosis (lymphocytolysis) was noted in all treated groups compared to placebo and sham control groups. The sponsor considered these changes to be a result of the pharmacological action of the test compound, which is an immunosuppressive agent. This is a reasonable explanation for the observed effects of FR900506 ointment on the thymus in rats.

Treatment related effects were noted in the lymph nodes. An increased incidence of sparse germinal centers in the cervical lymph nodes was observed for rats of both sexes treated with 0.5% FR900506 ointment (males: 15/20; females: 14/20). An increase in apoptosis in germinal centers was noted for four males in the 0.5% FR900506 ointment group.

Treatment related effects were noted in the spleen. Sparse peri-arteriolar lymphoid tissue in the spleen was recorded for four male rats and three female rats treated with 0.5% FR900506 ointment.

Treatment related effects were noted in the pancreas. Vacuolated islet cells in the pancreas were recorded for male and female rats treated with 0.3% and 0.5% FR900506 ointment (0.3% males: 12/20; 0.3% females: 5/20; 0.5% males: 10/20; 0.5% females: 13/20) with a low increase noted for males in the 0.1% FR900506 ointment group (2/20). Apparent atrophy/reduced numbers of islets were noted for male and female rats treated with 0.5% FR900506 ointment (males: 17/20; females: 4/20).

Treatment related effects were noted in the kidney. An increased incidence of mineralization at the corticomedullary junction in the kidney was seen for male rats treated with either 0.3% or 0.5% FR900506 ointment (0.3%: 8/20; 0.5%: 14/20). Mineralization was more common in female rats but there was little evidence of a dose relationship (sham: 9/20; placebo: 11/20; 0.03%: 14/20; 0.1%: 15/20; 0.3%: 14/20; 0.5%: 15/20). A dose dependent increase in cortical tubular vacuolation was seen for male and female rats treated with 0.1%, 0.3% and 0.5% FR900506 ointment (0.1% males: 2/20; 0.3% males: 11/20; 0.5% males: 18/20; 0.1% females: 5/20; 0.3% females: 11/20; 0.5% females: 15/20). An increased incidence of cortical tubular basophilia was observed in male and female rats in the 0.5% FR900506 ointment group (males: 18/20; females: 10/20).

Reduced seminal colloid was seen in the seminal vesicles from some male rats in the 0.3% and 0.5% FR900506 ointment groups. Acinar atrophy was seen in the salivary glands of male and female rats treated with 0.5% FR900506 ointment. Dilation of the lumen was seen at various levels of the gastrointestinal tract of rats in the 0.3% and 0.5% FR900506 ointment groups. An increased incidence of prominent adipose tissue was detected in the bone marrow of the femur for rats treated with 0.5% FR900506 ointment. Inflammation and degenerative changes were noted in the brain and spinal cord of rats treated with 0.3% and 0.5% FR900506 ointment.

- **Toxicokinetics** A summary of the plasma levels (mean) is provided in the following table.

Dose	Day 1-6 hr (ng/ml)	Day 1-24 hr (ng/ml)	Week 13-6 hr (ng/ml)	Week 13-24 hr (ng/ml)	Week 26-6 hr (ng/ml)	Week 26-24 hr (ng/ml)
0.03%	0.72	0.07	0.14	0.07	0.48	0.24
0.1%	0.77	0.31	0.63	0.61	3.1	0.48
0.3%	1.2	1.0	1.3	0.9	3.4	2.1
0.5%	1.8	1.7	4.3	1.6	5.0	2.8

The blood levels of FR900506 increased with increasing dose of FR900506 ointment. In general, the blood concentrations on week 13 and 26 were higher than on day 1. It is difficult to make any conclusions from pharmacokinetic data where only two time points were obtained after dose administration. It is not possible to calculate either C_{max} or AUC values from two time points. It would have been a more useful experimental design to take several time point samples over a 24 hr time point after dose administration. This experimental design would have allowed for the calculation of either C_{max} or AUC values for the various dose levels. The C_{max} and/or AUC values would have provided more useful toxicokinetic data for comparison of target organ toxicity in rats versus in humans. However, this data does support that significant systemic absorption did occur in this study which explains the wide range of target organ toxicity expressed in this 26 week repeat dose toxicity study.

Key Study Findings:

No treatment related dermal reactions were observed macroscopically at the treated skin sites. However, microscopic examination of the treated skin site revealed an increased incidence of epithelial hyperplasia/acanthosis for male rats receiving 0.1 or 0.03% FR900506 ointment. There was no dose response relationship observed for this effect and this epidermal response may be attributed to a mild irritancy of the FR900506 ointment, which was not visible macroscopically. The microscopic epidermal effect was not observed in female rats treated with the test compound. The remaining findings in this study were considered due to systemic action of FR900506 as a consequence of absorption through the skin.

Topical treatment of rats with FR900506 ointment resulted in pathologies in the thymus, spleen, cervical lymph nodes, bone marrow and decreased white cell counts (mainly lymphocytes). The effects of FR900506 on the pancreas and kidneys that were seen in this study have been well documented in previous systemic toxicity studies of FR900506. Treatment related mortalities occurred among rats in the 0.5% and 0.3% FR900506 ointment groups. Clinical signs of toxicity (particularly body tremors and signs indicative of poor clinical condition) were recorded for the animals that died in this study and also for other rats surviving treatment in the 0.5% and 0.3% FR900506 ointment groups. The sponsor considered these mortalities and clinical signs to be a result of inflammatory and degenerative changes seen microscopically in the brain and spinal cord. In addition, the sponsor considered these findings to be a secondary consequence of the immunosuppressant action of FR900506 and was

consistent with an infectious state attributable to latent viral activity. This appears to be an adequate explanation for these results.

The toxicity profile exhibited in this study was similar to that observed in previous subchronic or chronic oral toxicity studies performed in rats and mice. The toxicokinetic data support a substantial cutaneous absorption from the FR900506 ointment in rats. The mean FR900506 blood concentration at 6 hours post application of the 0.5% ointment (10 mg/kg/day) was 4.2 ng/ml and 5.0 ng/ml at week 13 and week 26, respectively. For comparison purposes, the mean FR900506 blood concentration for a 10 mg/kg/day dose in a 13 week oral (dietary admix) toxicity study in mice was 7 - 8 ng/ml. The toxicological profile in the chronic oral administration studies performed in mice and rats was quite similar to that outlined above for the 26 week topical toxicity study performed in rats.

The systemic toxicity results in this study mimicked the toxicity profile observed after systemic administration of FR900506 and can be attributed to the immunosuppressive nature of the compound. The systemic toxicity findings were apparent in a dose related manner in animals that received 0.5% and 0.3% FR900506 ointment and to a lesser extent in animals that received 0.1% FR900506 ointment. Therefore, the NOAEL in rats administered topical FR900506 ointment daily for 26 weeks was 0.03% (0.6 mg/kg/day; 3.6 mg/m^2/day) in this study.

Repeat Dose Toxicology Study #4:

13-week topical toxicity study of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs

Study Title:	13-week topical toxicity study of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs
Study No:	R97-0002-506-P2-E
Amendment #, Vol #:	000, 15-17
Conducting laboratory:	
Date of study initiation:	1/2/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

FR900506 ointment and placebo ointment were applied twice daily, seven days per week, for 13 weeks to an area equal to 40% of the total body surface area. The ointment from each application remained on the skin until the next application. The FR900506 or placebo ointment was applied to the test area by syringe at 2 µl/cm^2 and spread evenly with a rod. The treatment area was unabraided and unoccluded. Prior to application of each dose, residual test article was gently wiped off with gauze moistened with warm water. Application site hair was shaved on an as needed basis.

- *species/strain:* Yucatan micropigs
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 2-3 months
- *weight:* 6.8-15.1 kg males; 5.4-16.3 kg females

- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (mg/kg/day)	Number of Main Study Animals	
		Males	Females
Untreated Control	0	4	4
Placebo Control	0	4	4
0.1% FR900506 ointment	0.6	4	4
0.3% FR900506 ointment	1.8	4	4
1% FR900506 ointment	6	4	4
3% FR900506 ointment	18	4	4

Note: This dose range was selected for this study because the FR900506 ointment concentrations encompass 1X, 3X and 10X the ointment concentration dose range that was proposed for use in Phase 2 and 3 clinical studies. This was deemed an acceptable dose range for the 13 week micropig dermal toxicity study.

The dose level (mg/kg/day) was not provided by the sponsor. My calculation for this estimate is as follows (example calculated for the 0.1% FR900506 ointment). TBSA of a micropig is equal 0.74 m^2. The area of application is 40% of TBSA which equals ~0.30 m^2. Application rate equaled 2 $\mu l/cm^2$, which is approximately equal to 2 mg/cm^2 of ointment. Amount of ointment applied each time equals 6 gm (2 mg/cm^2 x 10000 cm^2/m^2 x 0.30 m^2). Dose per application of 0.1% FR900506 ointment equals 0.3 mg/kg (6 gm x 0.001 ÷ 20 kg). Daily dose of 0.1% FR900506 ointment equals 0.6 mg/kg/day (0.3 mg/kg x 2 applications/day).

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 711750K
0.1% FR900506 ointment – lot#711950K
0.3% FR900506 ointment – lot# 712050K
1% FR900506 ointment – lot# 71295XK, 71405YK and 702262K
3% FR900506 ointment – lot# 71305XK, 71415YK and 702362K

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily prior to next dose administration for the first 4 weeks and then on a weekly basis
- *Body weights:* weekly

- *Food consumption:* daily
- *Ophthalmoscopy:* prior to treatment and prior to necropsy
- *Hematology:* prior to initiation of treatment, during weeks 6 and 13
- *Clinical chemistry:* prior to initiation of treatment, during weeks 6 and 13
- *Urinalysis:* prior to initiation of treatment, during weeks 6 and 13
- *Gross pathology:* at sacrifice
- *Organs weighed:* heart, lungs, adrenal glands, parathyroid glands, pituitary gland, thyroid gland, liver, salivary gland, epididymides, seminal vesicles, testes, prostate gland, ovaries, uterus (with cervix), kidneys, spleen, thymus and brain
- *Histopathology:* The following organs were preserved in 10% buffered formalin: heart, aorta, larynx/pharynx, lungs, trachea, adrenal glands, parathyroid glands, pituitary gland, thyroid gland, large intestine (cecum, colon, rectum), liver, gallbladder, pancreas, salivary gland, small intestine (duodenum, jejunum, ileum), stomach, esophagus, tongue, epididymides, seminal vesicles, testes, prostate gland, ovaries, uterus (with cervix), vagina, kidneys, urinary bladder, bone marrow (femur), lymph nodes (mandibular, mesenteric), spleen, thymus, skin (treated and untreated), mammary gland, skeletal muscle, femur, sternum, grain (brain stem, cerebellum, cerebral cortex), spinal cord, sciatic nerve, eyes and gross lesions.

  All tissues and organs were examined for the untreated control, placebo control and all treatment groups.

- *Toxicokinetics:* Blood samples were obtained from toxicokinetic animals on day 1 and weeks 7 and 13. On day 1, blood samples were obtained at 0, 1, 2, 3, 6, 8 and 24 hours. During week 7, blood samples were obtained at 0, 3, 8 and 24 hours. During week 13, blood samples were obtained at 0, 1, 2, 3, 6, 8 and 24 hours.

Results:

- **Clinical signs** There was one mortality observed during this study. A 0.1% FR900506 ointment treated male was euthanized moribund on Day 82. Prior to euthanasia this animal had cutaneous erythema (Grades 1 or 2) and edema (Grades 1 or 2), cyanosis, labored breathing, papules, steeling and tremors. The animal was cold to the touch and prostrate. The animal had a reduced feed consumption and had lost weight. Gross necropsy revealed red fluid in the abdominal cavity; red discoloration of the mesenteric lymph nodes, stomach and jejunum; and red foci on the ileum and rectum. The contract lab states that this death was not attributed to the topical administration of FR900506 ointment. However, the contract lab did not specify the reasons why

they came to this determination. Therefore, it can not be ruled out that this death was not related to the test article administration. There were no other deaths that occurred during this study. No treatment related clinical signs were noted in any of the other animals.

- **Local dermal signs** Dermal effects were observed in the treatment site, but many of these dermal effects were seen in the ointment vehicle group and showed no clear dose response effect. A summary of the observed dermal effects follows. Skin papules occurred in 0, 8, 7, 6, 5, and 6 micropigs in the 0 (untreated), 0 (Vehicle), 0.1%, 0.3%, 1% and 3% groups, respectively. The mean papule onset for the males was 10, 6, 7, 5 and 3 weeks and for the females was 9, 6, 5, 4 and 10 weeks in the 0 (Vehicle), 0.1%, 0.3%, 1% and 3% groups, respectively. The papules were recorded as sites of edema and erythema according to the primary skin irritation criteria because the papules were often raised and red. Erythema (Grade 1 and 2) occurred in 7, 6, 5, 6 and 6 animals and edema (Grade 1 and 2) occurred in 3, 6, 5, 5, 2 animals in the 0 (Vehicle), 0.1%, 0.3%, 1% and 3% groups, respectively. The study director states that "The occurrences of papules and primary skin irritancy (i.e., erythema and edema) were not attributed to the topical administration of the test article because: 1) the frequency of occurrence and the mean onset values in the FR900506 ointment-treated groups were comparable to the values in the 0 (vehicle) group; and 2) the frequency of occurrence and mean onset values were not dependent on the FR900506 ointment concentration." I would concur with this conclusion based on the results from this dose range finding study.

- **Body weights** No treatment related effects on body weight were noted in this study.
- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Ophthalmoscopy** No treatment related ophthalmic examination findings were noted in this study.
- **Hematology** No treatment related hematologic findings were noted in this study.
- **Clinical chemistry** No treatment related clinical chemistry findings were noted in this study.
- **Urinalysis** No treatment related effects on urinary parameters were noted in this study.
- **Organ weights** No treatment related effects on organ weights were noted in this study.

- **Gross pathology** Pale or dark foci and crusts were noted at the administration site with a comparable incidence in the vehicle ointment groups and the 0.1%, 0.3% and 1.0% FR900506 ointment groups. No gross observations were noted at the administration site in the 3.0% FR900506 treated group.

- **Histopathology**

Focal acanthosis (3/4) and hyperkeratosis (2/4) of the skin at the administration was most prominent in 0.1% FR900506 ointment treated male animals. No other administration site observations in males of the other treatment groups occurred at an incidence greater than 1/4. No microscopic findings in the administration site skin were noted in untreated male animals. Chronic inflammation of the dermis was seen with comparable incidence and severity in placebo and FR900506 ointment treated female animals but was not noted in untreated female animals. The histopathological effects noted in this study is probably related to vehicle as opposed for FR900506.

No other treatment related histopathogical effects were noted in this study.

- **Toxicokinetics** A summary of the toxicokinetic (mean ± SD) parameters is provided in the following table. Pharmacokinetic parameters were calculated for day 1 and week 13 and are presented below. Only plasma levels are available for week 7 and they are not presented in this review.

Tacrolimus Conc. (% w/w)	Sex	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{0\text{-}24\ hr}$ (ng·hr/ml)	
		Day 1	Week 13	Day 1	Week 13	Day 1	Week 13
0.1	Male	0.8 ± 0.7	2.1 ± 1.6	2.0 ± 0.8	2.5 ± 2.4	4.6 ± 2.5	12.1 ± 4.9
	Female	0.3 ± 0.1	1.0 ± 0.7	3.3 ± 3.2	2.8 ± 0.5	2.5 ± 1.3	11.0 ± 4.0
0.3	Male	1.3 ± 0.6	1.4 ± 1.6	3.0 ± 2.4	4.5 ± 1.7	9.8 ± 5.8	11.9 ± 4.5
	Female	0.6 ± 0.6	1.3 ± 1.2	12.0 ± 13.9	3.3 ± 1.9	3.2 ± 3.1	11.0 ± 7.7
1.0	Male	5.2 ± 4.9	8.9 ± 14.1	8.0 ± 10.7	7.3 ± 1.2	52.2 ± 39.4	86.9 ± 130.4
	Female	0.9 ± 0.5	7.3 ± 4.9	7.8 ± 10.9	3.5 ± 3.0	13.5 ± 8.4	61.3 ± 45.7
3.0	Male	5.7 ± 2.8	27.8 ± 45.3	10.3 ± 9.7	7.0 ± 11.3	49.3 ± 23.7	62.1 ± 54.4
	Female	1.1 ± 0.7	7.2 ± 6.2	6.3 ± 3.5	2.5 ± 0.6	11.6 ± 4.5	42.3 ± 19.8

FR900506 was absorbed into systemic circulation following topical ointment administration to micropigs. The peak concentration in plasma was reached between 2 – 12 hours post dose. $AUC_{0\text{-}24\ hr}$ levels increased in a dose dependent manner that appeared to plateau between the 1.0% and 3.0% FR900506 ointment dose levels. AUC values increased 2-3 fold from Day 1 to Week 13 indicating a potential for drug accumulation after repeat dose administration. No apparent sex difference in systemic absorption was noted in this study.

Key Study Findings:

The only treatment related effect noted in this study was dermal reactions that exhibited a similar incidence and severity for vehicle ointment treated and FR900506 ointment treated animals. Histopathological findings in the treatment site skin (papules with corresponding acanthosis and hyperkeratosis) were noted with about the same incidence in vehicle ointment and FR900506 ointment treated animals. Therefore, the dermal effects noted in this study are probably related to vehicle ointment rather that FR900506. No other possible treatment related findings were noted in this study. Therefore, the NOAEL in micropigs administered topical FR900506 ointment twice daily for 13 weeks was 3.0% (18 mg/kg/day; 486 mg/m^2/day; Week 13 $AUC_{0\text{-}24\ hr}$ = 52.2 ng·hr/ml) in this study.

Repeat Dose Toxicology Study #5:

52-week topical toxicity of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs

Study Title:	52-week topical toxicity of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs
Study No:	96-0087
Amendment #, Vol #:	000, 18-20
Conducting laboratory:	[redacted]
Date of study initiation:	10/22/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

FR900506 ointment and placebo ointment were applied twice daily, seven days per week, for 13 weeks to an area equal to 40% of the total body surface area. The ointment from each application remained on the skin until the next application. The FR900506 or placebo ointment was applied to the test area by syringe at 2 µl/cm^2 and spread evenly with a rod. The treatment area was unabraided and unoccluded. Prior to application of each dose, residual test article was gently wiped off with gauze moistened with warm water. Application site hair was shaved on an as needed basis.

- *species/strain:* Yucatan micropigs
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 5-8 weeks
- *weight:* 2.7-7.3 kg males; 3.5-7.6 kg females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (mg/kg/day)	Number of Main Study Animals	
		Males	Females
Untreated Control	0	5	5
Placebo Control	0	5	5
0.03% FR900506 ointment	0.18	5	5
0.1% FR900506 ointment	0.6	5	5
0.3% FR900506 ointment	1.8	5	5
1% FR900506 ointment	6	5	5
3% FR900506 ointment	18	5	5

Note: This dose range was selected for this study because the FR900506 ointment concentrations encompass 1X, 3X and 10X the ointment concentration dose range that was proposed for use in Phase 2 and 3 clinical studies. This was deemed an acceptable dose range for the 52 week micropig dermal toxicity study.

The dose level (mg/kg/day) was not provided by the sponsor. My calculation for this estimate is as follows (example calculated for the 0.1% FR900506 ointment). TBSA of a micropig is equal 0.74 m^2. The area of application is 40% of TBSA which equals ~0.30 m^2. Application rate equaled 2 $\mu l/cm^2$, which is approximately equal to 2 mg/cm^2 of ointment. Amount of ointment applied each time equals 6 gm (2 mg/cm^2 x 10000 cm^2/m^2 x 0.30 m^2). Dose per application of 0.1% FR900506 ointment equals 0.3 mg/kg (6 gm x 0.001 ÷ 20 kg). Daily dose of 0.1% FR900506 ointment equals 0.6 mg/kg/day (0.3 mg/kg x 2 applications/day).

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 707865K, 710167K, 710267K, 701772K, 707378K
0.03% FR900506 ointment – lot# 707965K, 710367K, 701872K, 707478K
0.1% FR900506 ointment – lot# 708065K, 710467K, 701572K, 707578K
0.3% FR900506 ointment – lot# 708165K, 710567K, 701672K, 707678K, 710667K
1% FR900506 ointment – lot# 713168K, 713268K, 71616XK, 71626XK, 71636XK, 702773K, 702573K, 702673K
3% FR900506 ointment – lot# 713468K, 713568K, 71646XK, 71656XK, 71666SK, 703873K, 703773K

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* mortality - twice daily; clinical observations - weekly

- *Local dermal signs:*	daily prior to next dose administration for the first 4 weeks and then on a weekly basis
- *Body weights:*	weekly
- *Food consumption:*	daily
- *Ophthalmoscopy:*	prior to treatment and prior to necropsy
- *Hematology:*	prior to initiation of treatment, at months 3 and 6 and prior to necropsy
- *Clinical chemistry:*	prior to initiation of treatment, at months 3 and 6 and prior to necropsy
- *Urinalysis:*	prior to initiation of treatment, at months 3 and 6 and prior to necropsy
- *Gross pathology:*	at sacrifice
- *Organs weighed:*	adrenal glands, brain (brain stem, cerebellum and cerebral cortex), epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroid (with parathyroids) and uterus
- *Histopathology:*	The following organs were preserved in 10% buffered formalin: adrenal glands, aorta, bone marrow (femur), brain (brain stem, cerebellum and cerebral cortex), cervix/vagina, epididymides, esophagus, eyes, femur, gallbladder, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, ileum, jejunum), kidneys, larynx/Pharynx, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland, ovaries, pancreas, pituitary gland, prostate gland, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin (treated and untreated), spinal cord, spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina and gross lesions All tissues and organs were examined for the untreated control, placebo control and all treatment groups.
- *Toxicokinetics:*	Blood samples were obtained from toxicokinetic animals on day 1 and months 3 and 6 and prior to termination. On day 1, blood samples were obtained at 0, 1, 2, 3, 6, 8 and 24 hours. At months 3 and 6, blood samples were obtained at 0, 3, 8 and 24 hours. At month 12, blood samples were obtained at 0, 1, 2, 3, 6, 8 and 24 hours.

Results:

• **Clinical signs**	One male (0.1% FR900506 ointment) and one female (0.3% FR900506 ointment were found dead on day 365. In addition, one female (placebo ointment) was sacrificed moribund on day 187.

Necropsy findings suggested that all animal deaths were related to the blood collection procedure. No treatment related clinical signs were noted in this study.

- **Local dermal signs**

Dermal effects were observed in the treatment site, but many of these dermal effects were seen in the ointment vehicle group and showed no clear dose response effect. In males, dermal observations for all vehicle treated and test article treated groups included papules, circular purple ring, hyperpigmentation and hypopigmentation. The onset of papules ranged from day 42 for the 0.03% FR900506 ointment group to day 140 for the 3.0% FR900506 ointment treated group. The mean grade of severity of papules (severity = number of papules/animal) was not increased after week 25. The maximum severity of the papules in males ranged from grade 1 for the 0.3%, 1% and 3% FR900506 ointment groups to grade 2.4 for the vehicle ointment group. Neither the onset or the severity of the papules was related to dose level in males. The severity was greatest for males treated with vehicle only.

In females, the dermal observations for all vehicle treated and test article treated groups included papules, circular purples rings, hyperpigmentation and hypopigmentation. The onset of papules ranged from day 49 for 0.1% and 0.3% FR900506 ointment groups to day 119 for the 3% FR900506 group. The maximum severity of papules in females ranged from grade 1.0 for the 0.1% FR900506 ointment group to grade 2.75 for vehicle ointment treated animals. No papules, hyperpigmentation or hypopigmentation were noted in untreated males or females.

- **Body weights**

No treatment related effects on body weight were noted for male animals in this study. In females, body weighs for the 3.0% FR900506 group were significantly lower than for the untreated controls on weeks 19, 21-30, 32-34, 36, 37 and 39. The body weight gain was suppressed in 3.0% FR900506 ointment treated females almost throughout the dosing period. A graphical presentation of body weight data copied directly from the electronic NDA is provided below.

FIGURE 3
MEAN BODY WEIGHTS
MALE YUCATAN MICROPIGS

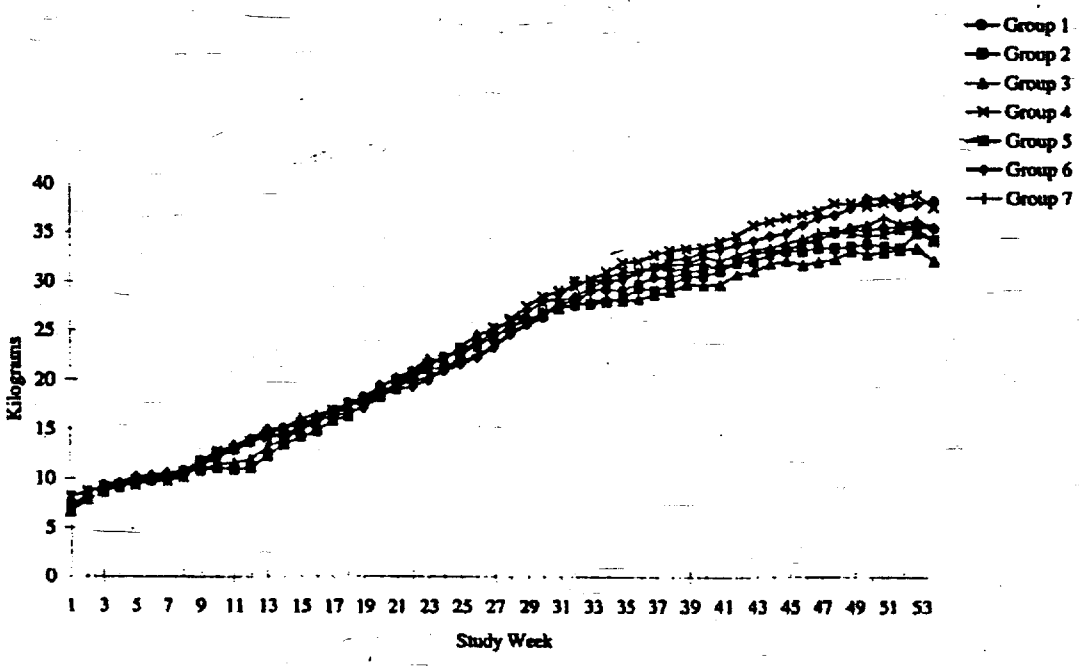
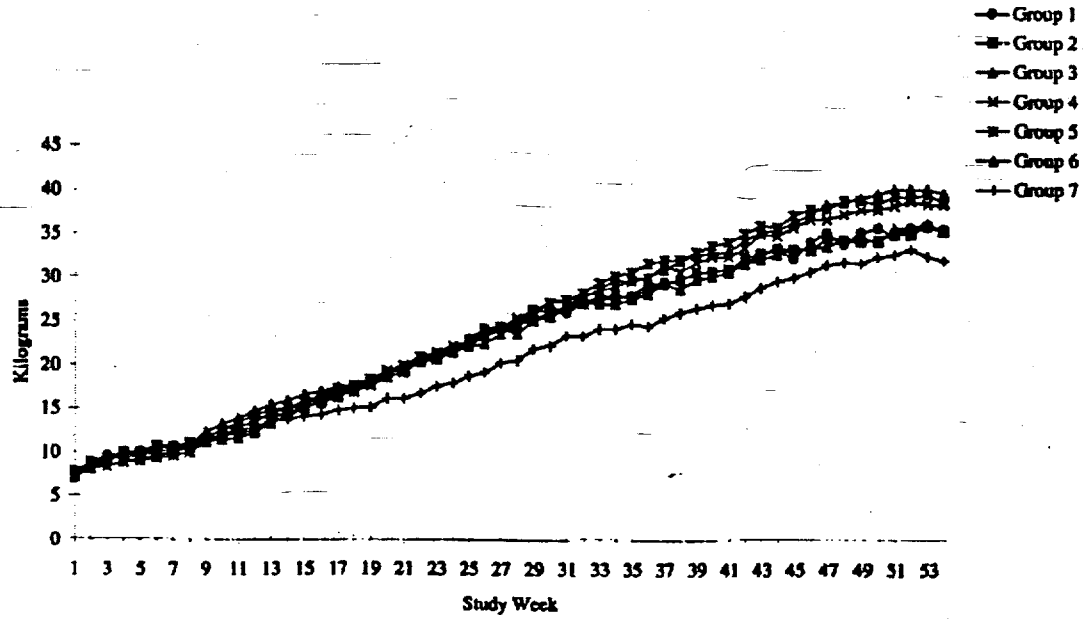


FIGURE 4
MEAN BODY WEIGHTS
FEMALE YUCATAN MICROPIGS



- **Food Consumption** No treatment related effects on food consumption were noted in this study.

- **Ophthalmoscopy** No treatment related ophthalmic examination findings were noted in this study.

- **Hematology** No treatment related hematologic findings were noted in this study.

- **Clinical chemistry** No treatment related clinical chemistry findings were noted in this study.

- **Urinalysis** No treatment related effects on urinary parameters were noted in this study.

- **Organ weights** No treatment related effects on organ weights were noted in this study.

- **Gross pathology** The administration site skin of several animals in vehicle ointment and FR900506 ointment treated groups contained areas of discoloration. These changes were single or multiple, varied in size, color and shape. Multifocal white discoloration one millimeter in diameter frequently occurred along the treated dorsum area in vehicle ointment and FR900506 ointment treated animals. Papules were noted at the administration site in a few females in all FR900506 ointment treated groups. No dose response was noted for this effect. Scabs and/or crusts were noted in vehicle ointment and FR900506 treated animals. No other possible treatment related macroscopic effects were noted in this study.

- **Histopathology**

Most of the skin lesions examined from male and female micropigs in vehicle ointment and FR900506 ointment treated groups were of similar morphology, which indicated a vehicle effect. Acanthosis and hyperkeratosis was noted at the administration site of most treated animals. Mononuclear cell infiltrates composed of lymphocytes with fewer histiocytes were present around vessels with the papillary (superficial) dermis. Sometimes eosinophils and neutrophils accompanied these infiltrates. A slight decrease in the incidence of these lesions were noted for male animals in the 1.0% and 3.0% FR900506 ointment groups. The incidence of these lesions was the same across all vehicle ointment and FR900506 ointment treated female animals. The intensity of these lesions ranged from minimal to moderate and showed no trends related to test article concentration.

No other treatment related histopathogical effects were noted in this study.

- **Toxicokinetics** A summary of the toxicokinetic (mean ± SD) parameters is provided in the following table. Pharmacokinetic parameters were calculated for day 1, month 3, month 6 and month 12. Pharmacokinetic parameters calculated for day 1 and month 3 are presented in the first table below and for month 6 and month 12 are presented in the second table below.

Tacrolimus Conc. (% w/w)	Sex	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{0-24\ hr}$ (ng·hg/ml)	
		Day 1	Month 3	Day 1	Month 3	Day 1	Month 3
0.03	Male	nc	1.2 ± 1.0	nc	5.4 ± 10.5	nc	11.7 ± 12.9
	Female	nc	0.4 ± 0.2	nc	16.0 ± 11.3	nc	7.1 ± 2.7
0.1	Male	0.5 ± 0.4	0.6 ± 0.2	6.8 ± 9.6	9.6 ± 8.8	2.4 ± 1.2	11.7 ± 4.1
	Female	3.4 ± 5.4	0.5 ± 0.1	5.6 ± 2.5	3.8 ± 4.0	29.0 ± 50.8	7.8 ± 1.9
0.3	Male	1.2 ± 0.4	1.4 ± 1.6	6.6 ± 9.8	6.4 ± 10.4	5.2 ± 2.0	17.8 ± 7.4
	Female	12.5 ± 18.4	0.8 ± 0.5	6.6 ± 2.2	2.8 ± 3.3	107.2 ± 177.6	10.8 ± 3.0
1.0	Male	10.8 ± 15.7	3.1 ± 1.7	20.8 ± 7.2	11.4 ± 11.5	97.4 ± 126.6	47.9 ± 24.4
	Female	1.2 ± 1.0	2.5 ± 1.4	7.8 ± 10.9	20.8 ± 7.2	13.5 ± 8.4	39.2 ± 18.8
3.0	Male	66.7 ± 41.6	7.1 ± 4.2	12.4 ± 10.8	11.8 ± 11.5	383.9 ± 300.5	106.2 ± 69.3
	Female	15.6 ± 18.2	5.0 ± 4.0	15.2 ± 12.1	14.4 ± 13.1	65.0 ± 55.6	86.4 ± 79.8

Tacrolimus Conc. (% w/w)	Sex	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{0-24\ hr}$ (ng·hg/ml)	
		Month 6	Month 12	Month 6	Month 12	Month 6	Month 12
0.03	Male	1.0 ± 0.6	0.6 ± 0.3	10.2 ± 8.0	3.0 ± 2.9	16.9 ± 7.7	10.0 ± 5.7
	Female	2.2 ± 3.7	0.6 ± 0.2	7.0 ± 10.0	1.4 ± 0.5	22.4 ± 33.1	5.7 ± 1.6
0.1	Male	2.7 ± 2.9	2.6 ± 1.3	4.8 ± 10.7	1.5 ± 1.3	29.3 ± 29.7	35.1 ± 26.8
	Female	0.7 ± 0.1	2.3 ± 1.1	2.2 ± 3.5	5.8 ± 1.8	10.5 ± 1.7	33.2 ± 12.5
0.3	Male	2.8 ± 1.4	4.4 ± 1.8	22.1 ± 7.2	7.6 ± 0.9	48.6 ± 24.4	58.5 ± 17.4
	Female	3.1 ± 1.4	5.1 ± 3.0	3.4 ± 2.9	6.0 ± 2.4	39.8 ± 17.7	80.4 ± 62.7
1.0	Male	7.8 ± 3.0	37.1 ± 41.4	7.0 ± 2.2	4.2 ± 3.6	123.7 ± 49.6	210.2 ± 73.3
	Female	8.8 ± 5.4	17.8 ± 18.2	17.6 ± 8.8	2.4 ± 2.3	167.8 ± 110.5	165.9 ± 113.5
3.0	Male	11.8 ± 4.4	127.6 ± 77.8	4.8 ± 4.4	7.0 ± 2.2	185.2 ± 86.4	1155 ± 811
	Female	14.4 ± 3.2	30.3 ± 9.0	8.2 ± 9.1	2.8 ± 3.0	243.8 ± 103.9	307.8 ± 187.5

FR900506 was absorbed into systemic circulation following topical ointment administration to micropigs. The peak concentration in plasma was reached between 2 – 24 hours post dose. Both C_{max} and $AUC_{0-24\ hr}$ levels tended to increase with the strength of FR900506 ointment. Due the large variability in blood levels of FR900506, it would appear there was not much potential for drug accumulation during the 12 month treatment period. No apparent sex difference in systemic absorption was noted in this study.

Key Study Findings:

Treatment related dermal effects were noted in this study that exhibited a similar incidence and severity for vehicle ointment treated and FR900506 ointment treated animals. The macroscopic changes included papules, circular purple ring, hyperpigmentation and hypopigmentation. Most of these changes corresponded microscopically to epidermal acanthosis with hyperkeratosis and perivascular mononuclear cell infiltrates in the papillary dermis

consistent with hyperplastic dermatitis. The histopathological findings in the treatment site skin were noted with about the same incidence in vehicle ointment and FR900506 ointment treated animals. Therefore, the dermal effects noted in this study are probably related to vehicle ointment rather that FR900506.

Significantly lower body weight was observed after week 19 for females in the 3.0% FR900506 ointment group. The reason for this decrease in body weight was unknown especially in view that food consumption for females at the highest dose level was similar to that of all other teat article treated groups. No significant changes in body weight were noted in the treated male groups. No other treatment related findings were noted in this study. Therefore, the NOAEL in male micropigs administered topical FR900506 ointment twice daily for 52 weeks was 3.0% (18 mg/kg/day; 486 mg/m^2/day; Month 6 $AUC_{0\text{-}24\ hr}$ = 185 ng·hr/ml) and for female micopigs the NOAEL was 1.0% (6 mg/kg/day; 162 mg/m^2/day; Month 6 $AUC_{0\text{-}24\ hr}$ = 168 ng·hr/ml) in this study.

Repeat Dose Toxicology Study #6:

13-week range finding phototoxicity and tolerance of tacrolimus ointment with UV radiation in hairless mice

Study Title:	13-week range finding phototoxicity and tolerance of tacrolimus ointment with UV radiation in hairless mice
Study No:	R96-0062-506-P2-E
Amendment #, Vol #:	000, 22
Conducting laboratory:	[redacted]
Date of study initiation:	1/2/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

This study consisted of two phases: Phase I was an Acute Photobiological Response study and Phase II was a 13 Week Range Finding Phototoxicity and Tolerance Study. Each phase will be described separately with corresponding results below.

- *species/strain:* Crl:SKH1-hr BR albino hairless mice
- *#/sex/group or time point:* Refer to dosing table below
- *age:* ~35 days
- *weight:* 11-28 g males; 12.6-25.7 g females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume. and infusion rate:* route = topical, for additional information refer to table below
- *source of irradiation (part I):* [redacted] compact arch high intensity solar simulator [redacted]
- *type of filter (part I & II):* 1 mm [redacted] glass filter

source of irradiation (part II): 6.5 KW xenon log arc water cooled burner [redacted]

The study design for Part I of the study is provided below. The objective of Part I was to determine if the interaction of either FR900506 or placebo ointment with UVR could elicit an acute photobiological response. Mice were lightly anesthetized and positioned on trays with masking tape. Test article was applied topically to 40% of the total body surface area. An aluminum foil mask was placed over each animal before UVR exposure. The mask had six holes, each with a diameter of 4 mm that served to define the irradiation sites. A single exposure of UVR was given to each of the irradiation sites. The radiant intensity of the source was checked at regular interval with a [redacted] meter. The durations of 0.5, 1.0, 1.4, 2.0 and 2.7 MEdD exposures were ~30, 60, 84, 120 and 162 seconds, respectively. A positive control (8-methoxypsoralen) and negative control (sunscreen) were incorporated into the study design.

Study Design – Part I

Treatment	# of male animals	UVR Exposure (MedD)[a]					
		0	0.5	1.0	1.4	2.0	2.7
Vehicle	5	+	+	+	+	+	N/A[c]
0.03% FR900506	5	+	+	+	+	+	+
0.1% FR900506	5	+	+	+	+	+	+
0.3% FR900506	5	+	+	+	+	+	+
1% FR900506	5	+	+	+	+	+	+
3% FR900506	5	+	+	+	+	+	+
8-MOP[b]	5	+	+	+	+	N/A[c]	N/A[c]
SPF4 SS[b]	5	+	+	+	+	+	+

a: MEdD refers to a UV dose adequate to elicit a barely perceptible response in skin; the range of doses delivered was thus 0 to 2.7 times MEdD.

b: 8-MOP: 8-methoxypsoralen in methanol (1 mg/ml): SFP 4 SS: Sun protection factor 4 sunscreen ([redacted] .

c: Not applicable

The study design for Part II of the study is provided below. The objective of Part II of the study was to determine the doses of FR900506 ointment that could be tolerated during a 52 week photocarcinogenicity study. Test article treated mice received 100 µl of test article topically applied to 40% of TBSA. Test article was applied across the posterior dorsal skin (back and sides) and each application remained on the skin for 24 hours. The application site was washed with a wet tissue prior to reapplication. The sequence of irradiation and topical FR900506 ointment application alternated from one irradiation day to the next. On Monday, Wednesday and Friday irradiation occurred after FR900506 application and on Tuesday and Thursday the irradiation preceded ointment application. There was ~60 min between dosing of the animals and UV irradiation or between UV irradiation and dosing of the animals. The UVR exposure, 272 R-B units per day, was based on the monitoring system that equates a minimal erythemal

dose (~20 mJ/cm^2) for humans (1000 R-B units). This level of irradiation is that used for induction of photocarcinogenicity. Side-to-side dividers were inserted in the cages prior to each irradiation period in order to maximize the delivery of the ultraviolet dose. Light intensity was monitored on representative racks by [redacted] dosimeters.

Study Design – Part II

Treatment	# of animals/sex/group	Approx Light (R/B units/day)	R-B units/week
Untreated (UVR) control	5	272	1360
Vehicle control	5	272	1360
0.03% FR900506	5	272	1360
0.1% FR900506	5	272	1360
0.3% FR900506	5	272	1360
1% FR900506	5	272	1360
3% FR900506	5	272	1360

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 711750K
0.03% FR900506 ointment – lot# 711850K
0.1% FR900506 ointment – lot# 711950K
0.3% FR900506 ointment – lot# 712050K
1% FR900506 ointment – lot# 71295XK, 714505YK
3% FR900506 ointment – lot# 71305XK, 71415YK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily – part I; weekly – part II
- *Body weights:* on day 1 and 4 – part I; weekly – part II
- *Gross pathology:* at sacrifice (part II only)
- *Histopathology:* Histopathological examination of the dorsal skin and all gross lesions was conducted for vehicle treated and all FR900506 ointment treatment groups (Part II only)

Results (Part I):

- **Clinical signs** No mortality was noted in this study. No treatment related clinical signs were noted in this study.

- **Local dermal signs** The average MEdD was calculated from the lowest UVR exposure (i.e., 0.5, 1.0, 1.4, 2.0 or 2.7 MEdD) required to elicit a cutaneous response for each animals in a group. FR900506 ointment at

concentrations as high as 3% had no effect on the average MEdD, which indicates that the test article had neither a phototoxic or photoprotective effect at the highest concentration of FR900506 ointment. The positive control, 8-MOP, elicited a response indicative of phototoxicity. The negative control, sunscreen, elicited a response indicative of photoprotection.

- **Body weights** A test article related body weight loss (e.g. 7.0% and 7.7% body weight loss in the 1.0 and 3.0% FR900506 dose groups, respectively) was observed during this study.

Results (Part II):

- **Clinical signs** Two, three and four male mice deaths occurred in the 0.3%, 1% and 3% dose groups, respectively. One and two female mice deaths occurred in the 1% and 3% dose groups, respectively. Clinical observations noted in male and female animals treated with 1% and 3% FR900506 ointment included emaciation, lethargy, tremors and hunched posture.

- **Local dermal signs** The dermal observations in this study indicated mild to moderate responses to the test article with a slight increase in severity for the highest (3%) dose group and a mild response elicited from the vehicle. All of the untreated control group animals had normal appearing skin on the last day of the experiment (Day 91). All of the mice in the treated groups had mild erythema and mild to moderate edema. The extent of erythema was not dependent on the dose, but the level of edema did appear to be dose dependent.

- **Body weights** Body weight loses occurred in all groups in both the males and females during the first week of the study. The body weight changes rebounded in all of the treated groups except for the highest dose (3% FR900506 ointment) in male and female mice and in the 1% FR900506 ointment dose group female mice. The first week weight loss may have been due to the stress to the animal with the initial handling since this effect was seen in both control groups. However, the body weight loss in the 3% dose group in male (↓7.8%) and female mice (↓20.1%) and in the 1% dose female mice (↓5.2%) compared to control mice after 13 weeks of treatment was probably related to the test article.

- **Gross pathology** The necropsy results for the mice that were found dead or euthanized moribund showed stomach, esophageal and abdominal cavity lesions in one, one and three males in the 0.3%, 1% and 3% dose groups, respectively. There were similar findings in one female from each of the same dose groups. The sponsor speculates that these findings are

related to the ingestion of the test article that probably occurred during preening. This is a plausible explanation for this result.

- **Histopathology** Histopathologic evaluation of the skin revealed a mild increase in the prominence of stratum granulosum and mild acanthosis of the epidermis in vehicle treated and FR900506 ointment treated mice. This finding did not exhibit a dose response relationship.

Key Study Findings:

Mortality was noted in Part II of this study. In part II of this study, body weight loss was noted in 3% FR900506 ointment treated males and females and 1% FR900506 ointment treated females. Mild erythema (not dose dependent relationship) and mild to moderate edema (dose dependent relationship) were noted in Part II of this study. A corresponding mild increase in the prominence of stratum granulosum and mild acanthosis of the epidermis, which was not dose dependent, was noted in vehicle and FR900506 ointment treated mice.

Based on the results of this experiment, the contract lab recommended that the highest dose for the 1 year photocarcinogenesis be the 1% FR900506 ointment. I concurred with this recommendation in my original review of this study when submitted to the IND (IND Serial 033; 7-16-96). The sponsor stated in the IND submission that the highest proposed dose in humans for the Phase 3 clinical trails would be the 0.1% dose. Therefore, the 1% dose in the photocarcinogenesis test would provide a 10X level above the proposed clinical dose.

Reproductive Toxicology Studies (data from NDAs 50-708/50-709):

The reproductive toxicity of tacrolimus was evaluated in Segment 1 (rats), Segment 2 (rats and rabbits) and Segment 3 (rats) studies[33,34,35,36]. Orally (gavage) administered tacrolimus altered reproductive function in female animals and reduced offspring viability during reproductive toxicity studies with rats (Segment 1 fertility; Segment 2 teratology; and Segment 3 perinatal and postnatal toxicity) and rabbits (segment 2 teratology). Male reproductive behavior was only slightly altered. The changes in reproductive parameters observed during these studies included increased copulatory intervals, decreased implantation, increased loss of fetuses, fewer births, and smaller litter sizes. No reduction in male or female fertility was evident. Adverse effects in offspring whose mothers received tacrolimus during pregnancy included markedly reduced viability and slightly increased incidence of malformation.

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[33] Study of FR900506 on fertility and general reproductive performance in rats. (1989) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR890455 (R89-0037-506-P2-E).

[34] Developmental toxicity study in rats (PO). (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0060-506-P2-E).

[35] Perinatal and lactation study of FR900506 in rats. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0073-506-P2-E).

[36] Teratology study of FR9000506 in rabbits. (1989) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R89-0036-506-P2-E).

The summary table for the reproductive toxicology profile of tacrolimus that was provided by the sponsor is reproduced below.

Study	Oral Dose (mg/kg/day)	Major Findings	
		Parental	F1 Offspring
Segment 1, rat	0.32	No observable effect	No observable effect
	1, 3.2	↓Body weight ↓Male copulatory index ↑Copulatory interval ↑Female diestrus period	Some lethality ↓Implantation ↑Post-implantation loss ↓Embryo/offspring viability
Segment 2, rat	1	No observable effects	No observable effects
	3.2	Some lethality ↓Body weight	↓Fetal weight ↑Post-implantation loss ↓Offspring viability ↑Skeletal variations
Segment 2, rabbit	<0.1	No observable effect	--
	0.1-3.2	--	No observable effect
	0.1, 0.32, 1	↓Body weight	↑Post-implantation loss ↓Viable fetuses ↑Morphological variations
Segment 3, rat	1	No observable effect	No observable effect
	3.2	↓Body weight	↓Body weight

Tacrolimus is labeled as a Pregnancy C category drug. Pregnancy category C would be appropriate for tacrolimus ointment for atopic dermatitis due to low systemic absorption.

The following information is contained in the Prograf® label:

No impairment of fertility was demonstrated in studies of male and female rats. Tacrolimus, given orally at 1.0 mg/kg (0.7-1.4X the recommended clinical dose range of 0.1-0.2 mg/kg/day based on body surface area corrections) to male and female rats, prior to and during mating, as well as to dams during gestation and lactation, was associated with embryolethality and with adverse effects on female reproduction. Effects on female reproductive function (parturition) and embryolethal effects were indicated by a higher rate of pre-implantation loss and increased numbers of undelivered and nonviable pups. When given at 3.2 mg/kg (2.3-4.6X the recommended clinical dose rage based on body surface area correction), tacrolimus was associated with maternal and paternal toxicity as well as reproductive toxicity including marked adverse effects on estrus cycles, parturition, pup viability and pup malformations.

In reproduction studies in rats and rabbits, adverse effects on the fetus were observed mainly at dose levels that were toxic to dams. Tacrolimus at oral doses of 0.32 and 1.0 mg/kg during organogenesis in rabbits was associated with maternal toxicity as well as an increase in incidence of abortions; these doses are equivalent to 0.5-1X and 1.6-3.3X the recommended clinical dose range (0.1-0.2 mg/kg) based on body surface area corrections. At the higher dose only, an increased incidence of malformations and developmental variations was also seen.

Tacrolimus, at oral doses of 3.2 mg/kg during organogenesis in rats, was associated with maternal toxicity and caused an increase in late resorptions, decreased numbers of live births, and decreased pup weight and viability. Tacrolimus, given orally at 1.0 and 3.2 mg/kg (equivalent to 0.7-1.4X and 2.3-4.6X the recommended clinical dose range based on body surface area corrections) to pregnant rats after organogenesis and during lactation, was associated with reduced pup weights.

No reduction in male or female fertility was evident.

There are no adequate and well-controlled studies in pregnant women. Tacrolimus is transferred across the placenta. The use of tacrolimus during pregnancy has been associated with neonatal hyperkalemia and renal dysfunction. Prograf should be used during pregnancy only if the potential benefit to the mother justifies potential risk to the fetus.

Since tacrolimus is excreted in human milk, nursing should be avoided.

Reviewer's Comments: No dermal reproductive toxicity studies were conducted with tacrolimus ointment. Therefore, it was recommended to the sponsor during a pre-NDA meeting to include the reproductive toxicity data contained in the Prograf® label in the Protopic® label. The multiple of human exposure will be revised in the Protopic® label (in the labeling review section below) based on the topical clinical dose for the atopic dermatitis indication.

Special Toxicology Studies (Submitted to the NDA):

Special Toxicology Study #1:

Local irritation study of FR900506 ointment in rabbits (II) (Primary dermal irritation)

Study Title:	Local irritation study of FR900506 ointment in rabbits (II) (Primary dermal irritation)
Study No:	R94-0096-506-P2-E; GLR940206
Amendment #, Vol #:	000, 10
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	1/28/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

The back of the animals was clipped and depilated longitudinally with a dipilatory (Eba Cream) the day before application of test article. The treatment area was divided into 4 5-cm squares and the alternate ones were abraded by making 4 epidermal incisions with an injection needle (23Gx1). FR900506 and placebo ointments (0.5 g of each) or 0.5 ml of the positive control (5% SLS) was applied to the abraded or intact skin sites, covered with surgical gauze and retained with adhesive tape for 24 hours. The entire trunk of each animal was wrapped in a stocking to hold the covers in position. The non-treated abraded or intact sites were not exposed

to any test article but were covered and wrapped in the same manner as treated animals. Twenty-four hours after application, the surgical gauze and adhesive tape were removed and the applied test article was removed with warm water.

Dosing:

- *species/strain:* male New Zealand White rabbits
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 13 weeks
- *weight:* 2.50-2.83 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Skin site	Dose (g/site)	Number of Male Animals
Non-treated Control	Intact	0	5
Non-treated Control	Abraded	0	5
Placebo Control	Intact	0.5	5
Placebo Control	Abraded	0.5	5
0.3% FR900506 ointment	Intact	0.5	5
0.3% FR900506 ointment	Abraded	0.5	5
1.0% FR900506 ointment	Intact	0.5	5
1.0% FR900506 ointment	Abraded	0.5	5
5% Sodium Lauryl Sulfate	Intact	0.5*	5
5% Sodium Lauryl Sulfate	Abraded	0.5*	5

* - 0.5 ml/site

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.3% FR900506 ointment – lot # 66953XK
1.0% FR900506 ointment – lot# 66873XK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Local dermal signs:* Erythema, eschar and edema were observed at the application site 30 minutes and 24, 48 and 72 hours after removal to the test article according to the Draize method.

Results:

- **Local dermal signs** No treatment related signs of dermal irritation were noted at either the intact or abraded treatment sites in placebo ointment, 0.5% or 1.0% FR900506 ointment treated animals. Positive control animals (5% SLS) showed signs of moderate to mild irritation.

Key Study Findings:

The dermal irritation potential of the 0.5% and 1% FR900506 ointments was considered to be very weak under the conditions of this study.

Special Toxicology Study #2:

Eye mucosa irritation study of FR900506 ointment in rabbits

Study Title:	Eye mucosa irritation study of FR900506 ointment in rabbits
Study No:	R94-0099-506-P2-E; GLR940207
Amendment #, Vol #:	000, 10
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	1/28/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Placebo or FR900506 ointment (0.1 g) was instilled into the conjunctival sac of the left eye of the animals and retained for 30 seconds. The right eye was not treated and served as a control to the treated eye.

Dosing:

- *species/strain:* male New Zealand White rabbits
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 14 weeks
- *weight:* 2.56-2.98 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (g/eye)	Number of Male Animals
Placebo Control	0.1	5
0.1% FR900506 ointment	0.1	5
0.5% FR900506 ointment	0.1	5

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.1% FR900506 ointment – lot # 66853XK
0.5% FR900506 ointment – lot # 66953XK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Eye mucosa observations:* Injuries to the cornea, iris and conjunctiva were observed macroscopically in each animal 2, 24, 48 and 72 hours after instillation. Changes were evaluated according to the Draize standard.

Results:

- **Eye mucosa observations**

No injuries to the cornea, iris or conjuctiva were noted in placebo ointment treated animals. No abnormal changes of the corneal epithelia were noted in the placebo ointment treated animals. Congestion (grade 1) was observed in the palpebral conjunctiva of 1 animal in the 0.1% FR90506 ointment group 2 hours after instillation. However, no injuries to the eye mucosa were noted in this animal at any observation time after this. No injuries to the eye mucosa were noted in the other 4 animals in the 0.1% FR900506 ointment group. No injuries to the cornea, iris or conjuctiva were noted in 0.5% FR900506 treated animals at any observation time.

Key Study Findings:

The eye irritation potential of the 0.1% and 0.5% FR900506 ointments was considered to be very weak under the conditions of this study.

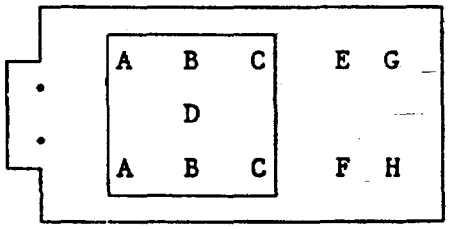
Special Toxicology Study #3:

Skin sensitization study of FR900506 ointment in guinea pigs (II)

Study Title:	Skin sensitization study of FR900506 ointment in guinea pigs (II)
Study No:	R94-0097-506-P2-E; GLR940208
Amendment #, Vol #:	000, 21
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	1/13/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

For the 1st induction, the upper back (about 2x4 cm area) of the animals was shaved the day before induction. Application sites for the study were outlined on the back. The diagram produced below for application site designations was copied from the electronic NDA.

Arrangement of application sites on the back



A, B & C: 1st induction sites
A: Water and FCA
B: FK506 oint., placebo oint. or DNCB
C: FK506 oint., placebo oint. or DNCB and FCA
D: 2nd induction site
E, F, G & H: Challenge sites
E: FK506 oint. or DNCB
F: Placebo oint. or acetone
G: DNCB
H: Acetone

0.05 ml of emulsion of equal volumes of water for injection and FCA was injected intradermally into each of sites A of all animals. 0.5% FR900506 ointment, placebo ointment and 0.1% DNCB acetone solution were injected into each of sites B in the FR900506 ointment, placebo ointment and positive control groups, respectively. Mixtures of equal volumes of 0.5% FR900506 ointment + FCA, placebo ointment + FCA and 0.2% DNCB acetone solution + FCA were injected into each of sites C in the FR900506 ointment, placebo ointment and positive control groups, respectively. In the negative control group, emulsion of equal volumes of water for injection and FCA was injected into each of sites C in the same manner and sites B were not treated.

For the 2nd induction, the back of the animals was shaved again 6 days after the 1st induction (the day before the 2nd induction) and 100 mg of vaseline containing 10% SLS was topically applied to site D without occlusion. The day of the 2nd induction, the vaseline was removed from the application sites by washing. The pretreatment was not performed in negative control animals. On the seventh day after the 1st induction, 200 mg (0.23 ml) of 0.5% FR900506 or placebo ointment was topically applied to site D in the respective FR900506 or placebo

ointment group. The treatment site was covered with surgical gauze and teflon sheet for 48 hours. An aliquot of 0.2 ml of 0.1% DNCB acetone solution was applied to the positive control animals in the same way. Negative control animals received no treatment.

For the challenge phase, the backs of the animals (a section different from the induction area) were shaved 13 days after the 2nd induction (the day before challenge) and divided into 2 or 4 parts (sites E, F, G and H; each circular area was a 2 cm diameter). For the challenge exposure, 100 mg (0.12 ml) each of 0.5% FR900506 and placebo ointments were topically applied to sites E and F, respectively, in the FR900506 and placebo ointment groups. The treatment sites were occluded for 24 hours in the same manner as that performed during the 2nd induction. An aliquot of 0.1 ml each of 1% DNCB acetone solution and acetone was applied to sites E and F, respectively, in the positive control animals. Negative control animals received 100 mg (0.12 ml) of 0.5% FR900506 and placebo ointments applied to sites E and F, respectively, in the same way and 0.1 ml of 1% DNCB solution and acetone was applied to sites G and H, respectively.

Dosing:

- *species/strain:* male Hartley guinea pigs
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 5 weeks
- *weight:* 305-368 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Induction		Challenge	Number of Male Animals
1st	2nd		
Water + FCA Placebo ointment + FCA Placebo ointment	Placebo ointment	FR900506 ointment Placebo ointment	20
Water + FCA FR900506 ointment + FCA FR900506 ointment	FR900506 ointment	FR900506 ointment Placebo ointment	20
Water + FCA DNCB + FCA DNCB	DNCB	DNCB Acetone	20
Water + FCA	Non-treated	FR900506 ointment Placebo ointment DNCB Acetone	20

FCA: Freund's complete adjuvant
DNCB: 1-chloro-2,4-dinitrobenzene

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.5% FR900506 ointment – lot # 66953XK
1-chloro-2,4-dinitrobenzene (DNCB; positive control) – lot # M3A9568
Freund's complete adjuvant (FCA) – lot # 33489JA

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Local dermal signs:* The skin of the application sites was observed before each induction and twice a week thereafter until challenge exposure. Erythema and edema were observed at the application site at 24, 48 and 72 hours from the start of the challenge application. Erythema and edema were scored according to the Draize method. A score of 2 or higher was regarded as a positive skin reaction.
- *Body Weights:* weekly

Results:

- **Local dermal signs**

Moderate erythema was observed at the applications sites in all placebo ointment treated animals 72 hours after the 2nd induction. No abnormal changes at any application sites were noted after challenge exposure with FR900506 or placebo ointment.

Slight erythema was observed at the application sites in FR900506 ointment animals in 6 animals and moderate erythema in 14 animals 72 hours after the 2nd induction. No abnormal changes were noted at any application sites after challenge exposure with FR900506 or placebo ointment.

Moderate erythema was observed in the positive group animals at the application sites in 16 animals and severe erythema in 4 animals 72 hours after the 2nd induction. Moderate erythema or severe erythema with edema was observed at the DNCB challenge sites in all the animals at 24 and 48 hours after the challenge exposure with 1% DNCB solution and acetone with mean scores of 2.05 and 2.00, respectively. Moderate erythema in 19 animals and slight erythema in 1 animal was noted at 72 hours after the challenge exposure (mean score: 1.95). At the acetone only exposure sites, moderate erythema was observed in 2 animals at 24 hours and slight erythema in 1 animal at 48 and 72 hours after challenge.

No abnormal changes in the skin after challenge exposure with FR900506 or placebo ointment was noted at any observation time in negative control animals.

- **Body Weight** No treatment related effect on body weight was noted for placebo ointment, positive and negative control animals. Bodyweight of the FR900506 ointment group was lower than that of the other groups until 7 days after the 1st induction. After the day 7, the body weight gain of the FR900506 ointment group was the same as that of the other groups.

Key Study Findings:

The results from the Magnusson and Kligman maximization test showed a positive response in DNCB sensitized animals after elicitation with DNCB. No skin reaction was noted after challenge treatment with 0.5% FR900506 ointment. Therefore, FR900506 ointment was considered to be a non-sensitizer in guinea pigs under the conditions of the study.

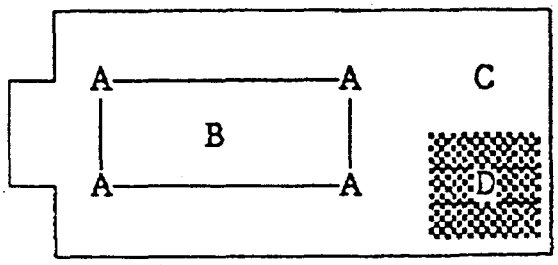
Special Toxicology Study #4:

Skin photosensitization study of FR900506 ointment in guinea pigs (II)

Study Title:	Skin photosensitization study of FR900506 ointment in guinea pigs (II)
Study No:	R94-0059-506-P2-E; GLR940244
Amendment #, Vol #:	000, 21
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	3/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

The cervical back of the animals was shaved 3 days before induction and an area about 2 x 4 cm was used as the application site for induction. The diagram produced below for application site designations was copied from the electronic NDA.

Figure 1: Arrangement of dosing sites



A: Injection site of water and FCA
B: Induction site
C: Challenge site
D: Challenge site covered

During the induction phase, 0.1 ml of emulsion of equal volumes of water for injection and FCA was injected intradermally into each of the 4 A sites. After stripping the stratum corneum of the application area with cellophane adhesive tape, 0.12 ml of 0.5% FR900506 or placebo ointment was applied topically without occlusion to the area (site B) using a 1 ml syringe. 0.1 ml of 1% TBS acetone solution (positive control) was given in the same manner. Except for the application site, the animals were covered with aluminum foil to protect from ultraviolet rays and restrained in a prone position. Thirty to 60 minutes later, 10 joules/cm^2 of ultraviolet rays were irradiated to the application site with a UVA irradiation apparatus [redacted] equipped with a glass filter to shut out the medium waves.

The animals were sensitized once a day for 5 consecutive days. FCA and water were injected intradermally only once on day 0.

During the challenge phase, the back of the animals was divided into 2 parts each 1.5 x 1.5 cm area along the midline and shaved 3 week after induction. An aliquot of 0.023 ml of FR900506 or placebo ointment was topically applied to sites C and D of the FR900506 or placebo ointment groups, respectively. An aliquot of 0.02 ml/site of 1% TBS acetone solution was given to positive control animals in the same manner. Site D of each animal was covered with aluminum foil to protect from UV exposure. UVA rays were administered to the animals under the same conditions as used during the induction phase.

Dosing:

- *species/strain:* male Hartley guinea pigs
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 5-6 weeks
- *weight:* 321-416 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Induction	Challenge	Number of Male Animals
Water + FCA Placebo ointment	Placebo ointment	20
Water + FCA FR900506 ointment	FR900506 ointment	20
Water + FCA TBA acetone solution	TBS acetone solution	10

FCA: Freund's complete adjuvant
TBS: 3, 4', 5-tribromosalicylanilide

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.5% FR900506 ointment – lot # 66953XK
3, 4', 5-tribromosalicylanilide (TBS; positive control) – lot # AP01
Freund's complete adjuvant (FCA) – lot # 33489JQ

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Local dermal signs:* The skin of the application sites was observed before each induction and twice a week thereafter until challenge exposure. Erythema and edema were observed at the application sites with and without UVA irradiation 24 and 48 hours after irradiation of the challenge phase sites. Erythema and edema were scored according to the Draize method.
- *Body Weights:* weekly

Results:

- **Local dermal signs**

Moderate erythema was observed at the application site in 1/20 placebo ointment treated animals on days 2 and 5 of induction and 1 week after the start of induction. No additional dermal effects were noted in placebo ointment treated animals up to the challenge phase. No dermal effects were noted in placebo ointment treated animals at any application site during the challenge exposure with placebo ointment alone or in combination with UVA irradiation.

Moderate erythema was observed at the application site in 1/20 FR900506 ointment treated animals on day 3 of induction. No additional dermal effects were noted in FR900506 ointment treated animals up to the challenge phase. No dermal effects were noted in FR900506 ointment treated animals at any application site during the challenge exposure with FR900506 ointment alone or in combination with UVA irradiation.

Moderate erythema was observed at the application sites in 2/10 TBS treated animals on day 3 of induction, in 3 animals on days 4 to 5 and in 1 animal 1 week after the start of induction. No additional dermal effects were noted in FR900506 ointment treated animals up to the challenge phase. Moderate erythema or erythema with edema was noted in 9/10 animals in the TBS treated group 24 and 48 hours after challenge exposure with TBS and exposure to UVA rays (mean score: 1.4 for the 24 and 48 hours observations). Moderate erythema was also observed on 1/10 animals 24 hours after challenge exposure with TBS without UVA irradiation (mean score: 0.1), but was not seen 48 hours after the challenge exposure.

- **Body Weight** No treatment related effect on body weight was noted for placebo ointment animals. Bodyweight of the FR900506 ointment group and positive control groups was slightly lower than that of the placebo ointment group from 7 and 21 days after the 1st induction, respectively.

Key Study Findings:

The results from the Adjuvant and strip photosensitivity test showed a positive response in TSB sensitized animals after elicitation with TSB and UVA radiation. No skin reaction was noted after challenge treatment with 0.5% FR900506 ointment and UVA radiation. Therefore, FR900506 ointment was considered to be a non-photosensitizer in guinea pigs under the conditions of the study. Unfortunately only UVA exposure was used in this study. The drug substance has absorption in the UVB range. Therefore, conduct of this study under UVA and UVB exposure would have been more beneficial.

Special Toxicology Study #5:

Effect of topical FR900506 (FK506, tacrolimus) on cutaneous pigmentation in normal dark Yucatan miniature swine

Study Title:	Effect of topical FR900506 (FK506, tacrolimus) on cutaneous pigmentation in normal dark Yucatan miniature swine
Study No:	R95-0167-506-P2-E
Amendment #, Vol #:	000, 23
Conducting laboratory:	Fujisawa USA, Inc., Research and Development, Deerfield, IL
Date of study initiation:	7/6/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()