CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-023

PHARMACOLOGY REVIEW(S)

NDA21-023

Review and Evaluation of Pharmacology/Toxicology data

Key Words: Topical Cyclosporine eye drop, Dry eye, Apoptosis, Acinar Epithelium, Lacrimal gland

Reviewer: Asoke Mukherjee

Division of Anti-inflammatory, Analgesic and Ophthalmic Drug Products

HFD-550

Review Completion Date: June 2, 1999

Review Number: One

NDA: 21-023

Serial Number, Date and Type of Submission: Serial # 1, Original NDA Application under 505 (b)1, Feb 24, 1999

Information to the sponsor: Yes ( ) No (X)

Sponsor: Allergan Inc. California 92623

Manufacturer of Drug Substance:

Drug: Cyclosporine ophthalmic emulsion

Code Name: AGN 192371

Generic Name: Cyclosporine Ophthalmic Emulsion, 0.05% preservative free.

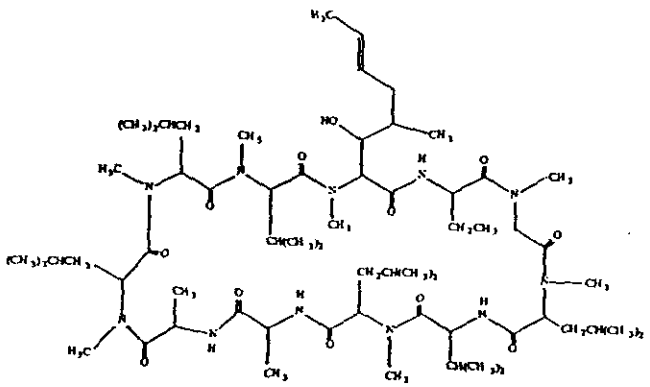
Trade Name: RESTASIS (proposed)

Chemical Name: Cyclo[[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl]

CAS Registry Number: 059865-13-3

Molecular Formula: C_{62} H_{111} N_{11} O_{12}. Molecular Weight: 1202.6

Structure:



Relevant NDA: [redacted] 50-733, [redacted], 50-574 and 50-573

Relevant IND: 32,133 and [redacted]

Relevant DMF: DMF [redacted] Type 1, DMF [redacted] type III

Drug Class: Immunosuppressant and immunomodulator.

Indication: [redacted]

Clinical formulation:

Cyclosporine, USP, 0.05% or 0.1% w/w
Castor oil PhEur, [redacted]
Polysorbate 80 NF
Carbomer 1342 NF
Glycerine USP,
Sodium hydroxyide USP,
Purified water USP,

Route of administration: Topical, instilled in eyes.

Proposed clinical use: The ophthalmic emulsion will be used topically for the treatment of KCS.

The proposed dose is: Ophthalmic drops 0.05% Cyclosporine, one drop in each eye, twice a day approximately 12 hours apart

Disclaimer: The sponsor submitted a letter of authorization from Novartis dated April 27, 1998 for cross-referencing all Cyclosporine INDs and NDAs.

Introduction and drug history:

Cyclosporine (CSA) is a cyclic polypeptide extracted from the fungus Beauveria nivea. It is an immunosuppressive agent approved for the treatment of the rejection of organ transplants, rheumatoid arthritis and psoriasis. Cyclosporine 0.2% ophthalmic ointment is approved for the treatment of chronic KCS in dogs. It CSA has also been investigated for several autoimmune and inflammatory diseases of the eye e.g. uveitis and Behcet's disease etc. The mode of action of CSA is through the inhibition of release of several cytokines e.g. IL-2, IL-3, IL-4, INF, GM-CSF and TNF. Most recently CSA has been shown to increase the transcription of TGF-β. In the present NDA the sponsor submitted study reports for the efficacy and safety of CSA for the treatment of keratoconjunctivitis sicca (dry eye) with or without Sjogren syndrome. Dry eye conditions result from reduced secretion of the tear from acinar epithelial cells in the eye. Experimental studies shown that invasion of T lymphocytes in the secretory gland affect the function of acinar epithelial.

The sponsor proposed that the local application of CSA would inhibit the T lymphocyte functions, reduce apoptosis of the epithelial, improve and restore the tear secretion.

Pharmacology:

The sponsor has submitted literature citations on the mechanism of actions of Cyclosporine and its effects in KCS in animal models. Summaries of some of these citations are presented in the review. However, the reviewer does not agree with the efficacy claims in the published papers for the indications not approved by the Agency.

Mechanism of action:

Page 011, vol 21:

The sponsor referred to several published papers on the immunosuppressive effect of Cyclosporine (CSA). However, the review written by Borel et al. Adv. Pharmacology 35, 115 provided a detailed analysis of its mode of action. CSA inhibits both humoral and cell mediated immunity. It is effective in chronic immune mediated inflammatory conditions and inhibition of graft rejection. The effect of CSA has been demonstrated in several inflammatory conditions including autoimmune uveitis, psoriasis, idiopathic nephrotic syndrom and rheumatoid arthritis.

CSA inhibits the function of T- lymphocytes without affecting the function of phagocytes or hemopoietic stem cells. The mechanism of CSA involves inhibition of cytokine release from the helper T cells. CSA binds with cytosolic protein known as cyclophilin. The CSA-cyclophilin complex inhibits calcium calmodulin-dependent protein calcineurine. Inhibition of calcineurine phosphatase activity by CSA-cyclophilin complex contributes to the inhibition of the function transcription factors e.g. NFAT and NFκB. The inhibition of the nucleotide regulatory factors results in the down regulation of the cytokine gene expression. CSA also has antagonistic effect on prolactin. It is suggested that inhibition of prolactin contribute to the antiinflammatory effect of CSA.

Drug activity related to proposed indication:

Page 239, vol 21:

The sponsor cited several published Papers on the effect of CSA in KCS in animals.

1. Role of apoptosis in the pathogenesis of canine keratoconjunctivitis sicca: The effect of topical Cyclosporine A therapy. Gao et al. Cornea, 17 (6), 654-663, 1998.

The authors stated that T lymphocytic infiltration was detected in the biopsies of lacrimal glands of dogs with spontaneous chronic idiopathic KCS. Apoptosis of the lymphocytes, lacrimal acinar epithelial and conjunctival epithelial cells were evaluated in spontaneous idiopathic KCS in dogs. The role of CSA in the apoptosis process has been discussed. Dogs that were clinically diagnosed with KCS were enrolled. Ten dogs with KCS were treated

with 0.2% CSA ophthalmic emulsion, three dogs with KCS were treated with the vehicle and another four normal dogs were used as the baseline control. Treated animals were dosed with one drop of CSA or the vehicle in each eye twice a day for 13 weeks. Lubricant eyedrops were applied at noon between the treatments. For the biopsy procedures, dogs were treated with atropine subcutaneously, anesthetized with i.v injections of Valium and Ketamine and treated with topical proparacaine. Doses of the treatment have not been mentioned. Biopsy specimens were obtained from the lacrimal glands and conjunctiva before and after 13 weeks of the treatment. Apoptotic cells were labelled and examined under light microscope. The apoptotic process was confirmed by DNA fragmentation analysis. Furthermore, monoclonal antibodies against fas-ligand, polyclonal antibodies against fas-ligand and monoclonal antibody against bcl-2 expressed proteins were used to show distribution of apoptotic receptor sites in the cell. Results indicated that the vehicle treated dogs had increase in the apoptosis of acinar, conjunctival epithelial cells and decrease in the apoptosis of the lymphocytes when compared to the normal dogs. The process was reversed after the treatment with CSA topically. It was concluded that suppression of apoptosis of lymphocytes and induction of apoptosis of epithelial cells of lacrimal glands and conjunctiva were the characteristic features of KCS in dogs. CSA treatment modulated the apoptic process.

Page 308, vol 21:

2. The sponsor has provided a review on KCS in dogs and the effect of CSA. The review was published by Kaswan and Salisbury, Vet clinics of North America, 20, 583, 1990. The review provided information covered by the citation described above. In addition, the review addressed the issue that the effect of CSA could be due to the irritant property of the drug. However, commonly known irritants are not effective in treating KCS. Therefore, the pharmacodynamic effect of CSA in KCS is not likely to be mediated by the irritant effect of CSA. The article also discussed about the opportunistic infections in the cornea and conjunctiva. However, the risk may be similar to corticosteroids. The issue of bioavailability of CSA in the lacrimal gland was raised in the publication. However, data on the CSA level in the lacrimal gland have not been provided.

Page 339, vol 21

3. Characteristics of a canine model of KCS: effective treatment with topical Cylosporine. By Kaswan. Lacrimal gland, tear film and dry eye syndromes, Edited by D.A. Sullivan, Plenum Press, N.Y 1994.

The review stated the mode of action of CSA for the treatment of KCS is as follows.

a. Modulation of cytokine production in the lacrimal gland.
b. Decreased recruitment of auto reactive lymphocytes from the conjunctiva to the lacrimal gland.
c. A direct neurohormonal effect of CSA mediated through prolactin receptors identified on lacrimal acinar epithelium. Prolactin is considered to be a natural ligand for cyclophilin. However, role of prolactin receptor in the lacrimal gland and its relationship to the effect of CSA is not clearly known.

The review also stated that CSA has a dose dependent side effect on hair growth and gingival hyperplasia.

4. Are dry eyes a sign of testosterone deficiency in women. Mamalis et al.Endocrine Soc. Intl. Mtg. 1996:849. Page 059, vol 22.

The abstract stated that dry eye syndrome is more prevalent in women. The authors hypothesized that KCS may be due to testosterone deficiency in women. In a population study, the serum concentrations of hormone precursors were determined from 26 women who had dry eyes and 19 matched control women.

The mean and SEM data for parameters are shown in the following table. The severity of dry eyes was determined from a questionnaire using a 4-20 scale.

Parameter	Unit	Patients (n=20)	Control (n=19)	P value
Age	yr	46.7 ±1.1	45.9 ± 1.2	NS
Severity	-	8.9 ± 0.5	5.0 ± 0.3	0.0001
Tear Osmolarity	mmol/L	308.3 ± 2.7	298.6 ± 2.2	0.012
Total Testosterone	ng/dL	25.6 ± 2.4	30.1 ± 3.0	0.238
Free Testosterone	pg/dl	2.5 ± 0.3	3.1 ± 0.5	0.282
SHBG	µg/dl	1.5 ± 0.2	1.9 ± 0.2	0.188
DHEA sulfate	µg/dl	61.8 ± 6.0	66.0 ± 7.1	0.66
Androstenedione	ng/dL	85.5 ± 6.7	95.8 ± 8.5	0.337
PG E_2	pg/ml	61.1 ± 10.3	93.9 ±19.7	0.123
Prolactin	ng/ml	8.7 ± 0.8	10.5 ± 0.8	0.131

The authors concluded that dry eyes in women resulted from testosterone deficiency. However, the testosterone levels between the patients and the control were not significantly different as shown in the above table.

5. An article entitled " Traffic of major histocompatibility complex class II molecules in rabbit lacrimal gland acinar cells", Mircheff et al. Invest. Ophthalmology & Visual Science, 35, 3943, submitted to the NDA page 075, vol 22.

The authors investigated the expression of MHC II molecules on acinar epithelial cells in the rabbit model. Using monoclonal antibodies, the authors showed that MHC II molecules are expressed in the cell surface of acinar cells. The MHC II molecule binds with the auto-antigen, presents to T lymphocytes and induces inflammatory changes. The article suggests that autoimmunity in the lacrimal gland contribute to the pathogenesis to dry eye syndrome.

6. Hormonal support of lacrimal function, primary lacrimal deficiency, autoimmunity and peripheral tolerance in the lacrimal gland. Mircheff et al. Ocular Immunology and Inflammation, 4, 145, 1996. Page 84, vol 22:

The review covered the pathogenesis of dry eye diseases.

Dry eye or KCS is the leading cause of patient visits to the eye care specialists other than the refractory correction. The clinical manifestation of dry eye ranged from contact lens intolerance to severe conjunctival inflammation and corneal damage. Lacrimal insufficiency could be immune related and non-immune related.

The immune-related lacrimal insufficiency results from Sjogren's syndrome, graft vrs host disease, diffuse infiltrative lymphocytosis syndrome of HIV (DILS) and sarcoidosis. Inflammatory cells e.g. CD_4 positive T-lymphocytes, CD_8 positive T-lymphocytes, B-lymphocytes, granulomatous macrophages etc. infiltrate lacrimal glands in above conditions. Some of the immune mediators impair the ability of the lacrimal secretory epithelium to regenerate.

Non-immune-mediated lacrimal insufficiencies are due to congenital alacrimia, sensory or secretomotor nerve damage, destruction of lacrimal secretory tissues and due to the side effects of number of drugs.
The authors also indicated the role of estrogen and androgen deficiency in the pathogenesis of dry eye syndrome.

7. Tear fluid influence on the ocular surface, Pflugfelder. S.C, Adv. Exp. Med. Bio, 438, 611-617,1998.

   Page 217, vol 22:

   The review stated that factors secreted in the tear fluid by the lacrimal gland are classified into:

   a. Antimicrobial factors e.g. lactoferrin, lysozyme and IgA
   b. Growth regulating factors e.g. EGF, TGF-α, TGF-β and vitamin A.

Release of these factors from acinar cells is stimulated by cholinergic nerve stimulation and can be blocked by muscarinic antagonists.

The tear fluid EGF concentrations decrease following induction of nasal-lacrimal reflex. The review suggests that elaborate homeostatic mechanisms exist to maintain the tear fluid EGF concentration within a range that may be optimal for the ocular surface epithelium.

Human tear fluid inhibits TGF-β sensitive growth of mink lung epithelium. Therefore, TGF-β is responsible for the antiproliferative activity of the tear fluid. Tear fluid maintains a low state of epithelial proliferation on the ocular surface under the normal condition. Under a disease condition as in KCS, reduction in the concentration or activity of TGF-β in the tear fluid could lead to conjunctival epithelial proliferation e.g. squamous metaplasia of the conjunctival epithelium. The functions of normal tear fluids are to inhibit conjunctival epithelial proliferation, to promote terminal differentiation, stimulation of epithelial membrane mucus production and promotion of goblet cell differentiation.
Ancillary Pharmacology studies: No ancillary pharmacology study reports have been provided.

Summary of Pharmacology:

KCS is associated with the immunological and non-immunological mechanisms. Increased infiltration of lymphocytes and degeneration of tear forming cells are the common causes of KCS. Expression of MHC II in the acinar epithelium and development of autoimmunity are contributory factors to the development of KCS. CSA ophthalmic emulsion is targeted for the suppression of the lymphocytic infiltration and autoimmunity of the lacrimal gland.

CSA is a known immunosuppressant approved for organ transplantation, RA and psoriasis in humans. It inhibits several cytokines. Among other mechanisms, CSA also contributes to the efficacy in the inflammatory condition by displacing prolactin from lymphocytes. Presence of prolactin receptors has been shown in the lacrimal gland. However, its direct role in the tear secretion in KCS is not known.

Effect of CSA in the KCS in dogs has been investigated. A published report suggests that CSA induces apoptosis of lymphocytes and inhibits apoptosis of acinar epithelial cells. The process contributes to the regeneration and differentiation of secretory epithelial cells in the lacrimal gland. Data from the literature also suggested that CSA induces growth of hair by its direct action in the hair follicle. The pharmacological effect can contribute to the growth of eyelashes.

The literature citations also indicated the role of endocrine hormones e.g. estrogen and testosterone in the pathogenesis of KCS. As with many other autoimmune diseases, testosterone deficiency could contribute to KCS. However, CSA does not have a direct effect on the steroid hormone receptors.

Tear fluids contain several growth factors among which TGF-β is important because of its contribution to the immunosupression to the eye, differentiation and function of lacrimal epithelial cells. A recent publication on the mode of action of CSA indicates that CSA induces TGF-β in vitro and in vivo (Hojo et al. Nature, 397, 530, 1999). TGF-β reduces epithelial proliferation in ocular tissues and contributes to the normal function of secretory cells in the eye.

CSA inhibits cytokine and growth factors that contribute to its efficacy for the treatment of KCS. Results of long term deprivation of these factors on the growth, differentiation and function of acinar cells are unknown. Therefore, consequence of long term treatment of KCS with CSA needs to be monitored in the clinical studies.

Safety pharmacology:

No study report has been submitted in the NDA. However, approved package insert of CSA indicates hypertension and nephrotoxicity are the major side effects of CSA. Exact mechanism of the cardiovascular side effects is not known. Published literature indicated the contributions of endothelin and thromboxane in the pathogenesis of CSA induced kidney toxicity and hypertension. The package insert also indicated hepatotoxicity, convulsions, hirsutism and gum hyperplasia as the side effects of CSA.

Borel et. al. Adv. Pharmacology, 35, 115, 1996, also suggested that the stimulation of hair growth (hypertrichosis) is a common side effect of CSA observed in humans and animals. The effect of the Immunosuppressive on hair growth may be unrelated to immunosuppression.

Studies reviewed in the submission:

1. A six-month ocular and systemic toxicity study with a two-month recovery period in New Zealand white rabbits. Report # 1793-2936-6. Page 001, vol 15. (see page 21)

2. A fifty-two week ocular and systemic study of Cyclosporine in dogs with an eight -week recovery period. Report # — 985-126. Page 001, vol 17. (see page 10)

3. A six-month interim toxicokinetic report: Pharmacokinetic analysis of Cyclosporine A in dog blood for study # —— 985-126, 52-week ocular and systemic study of Cyclosporine in dogs with an 8 week recovery period. Report PK-96-001, page 27, vol 19. Also submitted in NDA page 138, vol 18 (see page 17)

4. The blood to plasma concentration ratio of ^{3}H-Cyclosporine-A in mouse, rat, rabbit, dog and human in vitro. Report # PK-94-108, Page 187, vol 18. (see page 47)

5. Ocular pharmacokinetics of Cyclosporine after a single eye drop instillation of a 0.2% ^{3}H-cyclosporine ophthalmic emulsion into albino rabbit eyes. Study # PK-95-010, page 257, vol 18. (see page 42)

6. Investigation of ocular metabolism of Cyclosporine after a single drop instillation of a 0.2% ^{3}H cyclosporine ophthalmic emulsion into albino rabbit eyes. Study # PK-95-011, page 289, vol 18. (see page 58)

7. Pharmacokinetic kinetic analysis of Cyclosporine A in rabbit blood for study # 1793-2936-6 " AGN 192371-Cyclosporine ophthalmic emulsion: A six-month ocular and systemic toxicity study with a two month recovery period in New Zealand white rabbits". Study # PK-95-066, page 361, vol 18. (see page 37)

8. The effect of oil globule size on ocular absorption of ^{3}H-Cyclosporine after topical instillation of three 0.2% ^{3}H-cyclosporine oil-in-water emulsions into rabbit eyes. Report # PK-95-074, page 001, vol 19. (see page 60)

9. Dose proportionality of ocular tissue ^{3}H-Cyclosporine concentrations after a single dose administration of 0.05%, 0.2% and 0.4% ^{3}H-Cyclosporine emulsions into rabbit eyes. Report PK-96-011, Page 075, vol 19. (see page 48)

10. Ocular absorption and disposition in beagle dogs following multiple ocular doses of 0.2% ^{3}H Cyclosporine emulsion. Report PK-96-016, page 100, vol 19. (see page 49)

11. Ocular absorption and disposition in beagle dogs following single ocular doses of 0.2% ^{3}H Cyclosporine emulsion. Report PK-96-017, page 194, vol 19. (see page 55)

12. Pharmacokinetic analysis of Cyclosporine in dog blood for 52-week ocular systemic study of Cyclosporine in dogs with an 8-week recovery period. Report PK-96-023, page 298, vol 19. (see page 17)

13. Ocular Cyclosporine distribution during 9 ½ days of dosing of 0.05% and 0.1% ^{3}H-Cyclosporine A emulsions to albino rabbits. Report PK-98-074, vol 328, vol 19. (see page 46)

14. Cyclosporine a [redacted] special study. Report # PA-1998-010, page 002, vol 20. (see page 38)

15. A multicenter, double masked, randomized, vehicle-controlled parallel-group study of the safety and efficacy of Cyclosporine 0.05% and 0.1% ophthalmic emulsions used twice daily for up to one year in patients with moderate to severe Keratoconjunctivitis Sicca. Clinical report # 192371-002, page 240, vol 20. (see page 41)

Studies not reviewed in the submission:

[redacted]

General Comments: Nil

Study Title:

A fifty-two week ocular and systemic toxicity study of Cyclosporine in dogs with an eight-week recovery period.

Study #: 985-126.

Page 001,vol 17.

Conducting Laboratory and Location:

Date of the study initiation: March 9, 1995

GLP Compliance: The study was conducted in compliance with GLP regulation.

QA report: Yes

Methods:

Dosing:

Species/Strain	#/sex/group	Age (months)	Weight (kg)
Dog, Beagle	6	6-7	6.3-10.9 M, 5.8-9.1 F

Satellite group used for recovery:

Two animals per sex/group were sacrificed after 8 weeks of recovery period for groups 1,3 and 4. However, only one male was allowed to undergo 8 weeks of recovery in the group 2.

Dosage groups in administered units:

Group	Conc (%)	Male	Female	Drops/day
1, Control	0, Placebo for 0.4%	G31853-G31858	G31859-G631864	6
2, Low	0.1	G31865-G31870	G31871-G31876	3
3, Mid	0.2	G31877-G31882	G31883-G31888	3
4, high	0.4	G31889-G31894	G31895-G31900	6

Route, form, volume:

The drug product or the vehicle was administered as ocular drops in the left eye. The right eye served as the untreated control. The control and the high dose groups received six applications at 2-hour intervals. The remaining animals received three applications at 3-hour intervals. Animals were treated until the day before the terminal sacrifice. Each drop was approximately 40 µL in volume.

Drug, Lot #, formulation and vehicle:

Cyclosporine ophthalmic emulsion (AGN 192371)

Lot #	Concentration
1. 8746X-10619B	Placebo, pH 7.4
2. 8735X-10718	0.1%, pH 7.4, analyzed concentration 0.097%
3. 8734X-10813	0.2%, pH 7.3, analyzed concentration 0.192%
4. 8734X-10621B	0.2%
5. 8733X-10622B	0.4%
6. 8733X-10814	0.4%, pH 7.2, analyzed concentration 0.374%

Formulations of the drug product and vehicle for the study have not been provided in the report. However, chemistry review dated May 21, 1999, page 45 provided the preclinical formulations (see attachment).

Observations and times:

Clinical signs:

All dogs were observed twice daily for mortality and moribund conditions at least six hours between each observation. Each dog was observed once daily for the evidence of toxicity or side effects. A thorough physical examination was conducted for each dog once a week.

Body weights:

Weekly body weights of the animals were recorded prior to the treatment, during weeks 1-14 of the treatment and once every four-week thereafter. However, the body weight was recorded at three-week interval between weeks 50-53.

Food Consumption:

Food consumption was recorded weekly for 1-13 weeks and once every four week from weeks 13-49. Food consumption was recorded once between weeks 49-52.

Ophthalmic Examination:

Ocular discomfort and irritation were examined and scored before the first dose and before last dose on each day for the first week. Ocular discomfort was examined once weekly thereafter. Indirect ophthalmoscopic examinations were performed on days -8, 27, 90, 170, 268, 364 and 420, respectively (page 060, vol 17). Slit lamp examinations were conducted prior to the treatment, during weeks 4, 13, 25, 39, 52 and 60 (page 063, vol 17). Surviving animals were examined by slit lamp on week 62. One percent Mydriacyl was applied in both eyes before the examination. Fluorescein stain was used for each examination after week 4.

Blood pressure and ECG:

Indirect blood pressure and ECG were recorded prior to initiation of the treatment, on weeks 26 and 52.

Hematology:

Blood samples were collected from the jugular vein. Animals were fasted overnight prior to all sample collections. Following parameters were determined prior to the treatment and during weeks 13, 26, 39, 52. Samples were collected in week 62 from animals allotted for recovery.

Activated partial thromboplastin time, corrected leukocyte count, differential leukocyte count and cell morphology, erythrocyte count, hematocrit, hemoglobin, leukocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, platelet count and prothrombin time.

Serum Chemistry:

Blood samples were taken during the same time as indicated in the hematology section for the assay of following parameters:

Alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, gamma glutamyltransferase, globulin, glucose, total bilirubin, total protein and urea nitrogen.

Urine analysis:

Urine samples were collected in clean containers placed under the drainage opening of each cage. Samples were collected prior to the treatment, and during weeks 13, 26, 39, 52. Samples were collected on week 60 from animals allotted for recovery. Following parameters were examined.

Appearance/color, glucose, ketones, occult blood, protein and specific gravity.

Toxicokinetics:

Blood samples were collected from first three males and females in each group on day 7 and week 49 at following time points: prior to the first daily dose, 1, 5, 7, 9, 11, 12, 24 hours after the first daily dose. Also blood samples were collected (for the trough level) prior to the first daily dose on one day during weeks 12 and 26. . The samples were shipped to Allergan for the analysis.

Gross pathology:

After at least 52 weeks of the treatment, first four animals/sex /group were weighed and anesthetized with sodium thiopental and exsanguinated. Remaining animals were anesthetized and exsanguinated after 8 weeks of the recovery. At necropsy, the carcass, all external body orifices and visceral organs were examined. One male # G31869 from group 2 was sacrificed in the moribund condition during week 33.

Organ weights:

Following organs were weighed after removing the fat and other tissues.

Adrenals, epididymides, heart, ovaries, spleen, liver, kidneys, lungs, testes, thymus, thyroids, brain, pituitary gland.

Histopathology:

Following tissues were preserved in Histology sections were prepared by a standard method. Microscopic examinations were performed for all tissues at all doses of CSA and placebo treated animals. Sections were stained with stain.

Adrenals, aorta, sternum bone marrow, brain with brainstem, medulla, pons, cerebellar cortex and cerebral cortex, cervical spinal cord, colon, cecum, rectum, duodenum, jejunum, ileum, esophagus, eyes with optic nerves, femur including articular surface, heart, kidneys, liver with gall bladder, lumbar spinal cord, lungs and trachea, mammary gland, mesenteric lymph node, mid-thoracic spinal cord, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, skin, spleen, stomach, testes with epididymedes, thigh musculature, thymus, thyroid, trachea, urinary bladder, uterus, vagina and cervix.

Results:

Clinical signs:

There was no CSA treatment related clinical signs observed. However, there were incidences of lacrimation both in the placebo treated and Cyclosporine (CSA) treated eyes. Number of animals that showed lacrimation are shown in the following table.

Eye	Placebo	0.1%	0.2%	0.4%
	M F	M F	M F	M F
Both	2 2	1 2	3 1	4 3
Right (untreated)	3 0	0 0	1 0	1 0
Left (treated)	3 6	3 4	3 3	5 6

The data suggest that the lacrimation in the left eye could be placebo related. The untreated eye did not show lacrimation except male dogs in the placebo, 0.2% and 0.4% groups.

Male # 31869 treated with 0.1% CSA was sacrificed on week 33 for humane reasons.

Body weight:

There was a reduction in the body weight gain (kg) in treated animals during weeks 1 to 52. However, it was not statistically significant and considered to be incidental.

Group 1 (placebo)	Group 2 (0.1%)	Group 3 (0.2%)	Group 4 (0.4%)
M F	M F	M F	M F
2.3 1.7	2.0 1.1	1.6 1.4	1.7 1.4

Food consumption:

Mean food consumption (kg) and the standard deviation of the mean are shown in the following table.

Group 1 (placebo)	Group 2 (0.1%)	Group 3 (0.2%)	Group 4 (0.4%)
M F	M F	M F	M F
52.1± 5.3 41.0± 4.4	46.4 ± 7.4 40.4±4.8	44.2 ± 8.4 40.6±5.8	45.9 ±6.0 35.7± 2.0

Above table suggest that male and female dogs in group 4 had a reduced food consumption compared to the placebo group. However, it was not statistically significant and considered to be incidental.

Ophthalmic Examination:

Total number of positive conjunctival discharge scores in the left eye (treated eye) for the entire study period is shown in the table below. Figures in parentheses represent number of dogs that showed positive score.

	Placebo M F	0.1% CSA M F	0.2% CSA M F	0.4% CSA M F
Prior to 1st dose	51 (3) 121(5)	5(2) 47(3)	63(3) 4(2)	105 (5) 93(4)
After last dose	83(3) 178(6)	33(4) 110(6)	108(4) 47(5)	199(6) 201(6)

The score was given in 0-3 scale. 0= no discharge, 1= any amount different from normal, 2= discharge with moistening of the lids and hair, 3= discharge with moistening of the lid, hair and considerable area around the eye.

Above data suggest that the conjunctival discharge was noted in the placebo and CSA treated animals. The conjunctival discharge increased at 0.4% CSA compared to the placebo treatment. The composite score for the untreated eye is not provided in the result.

Number of animals affected by conjunctival redness in the left eye is shown below. These data were taken from page 57 and 59, vol 17.

	Placebo M F	CSA 0.1% M F	CSA 0.2% M F	CSA 0.4% M F
Predose, Left eye	1 1	2 4	2 2	5 2
Post Dose, Left eye	1 1	4 3	2 2	5 3

The redness was observed in all CSA treated animals. The prevalence of redness was greater in the 0.4% group. However, incidences in the placebo treated animals were smaller than the treated groups.

Indirect ophthalmoscopy and slit lamp examinations did not show compound related change in the cornea, lens and retina. Mild unilateral epiphora/lacrimation was observed in the male dogs in the treated eye. The incidences are shown below. Total number of dogs in each group was 6.

	Placebo M F	0.1% CSA M F	0.2% CSA M F	0.4% CSA M F
Lacrimation/ epiphora	1 3	2 3	1 3	4 5

Electrocardiography:

There was no abnormality in the ECG. Mean arterial blood pressure did not change significantly during the treatment period. Group mean values for heart rate were decreased at 0.4% CSA in the male animals. Female dogs did not show similar change in the heart rate. However, biological significance for the change is unknown. The data are shown in the following table.

Heart rate (b/min) in male dogs.

	Week -2	Week 26	Week 52
Placebo	131±16	127±22	140±18
0.1% CSA	121±20	122±20	132±46
0.2% CSA	133±13	106±23	108±16
0.4% CSA	124±20	110±32	94±23*

$p < 0.05$

Hematology:

Male dogs did not show treatment-related changes in the hematology parameters. Female dogs showed statistically significant reduction in the RBC, hemoglobin and hematocrit values at 0.4% dose on week 52. The changes were considered to be incidental due to a small magnitude. The data are shown in the following table.

	RBC 10 9/µL	Hb (g/dL)	HCT (%)
Placebo, (CSA 0%)	6.54	15.6	43.8
CSA 0.4%	6.00*	14.4*	39.8*

*P<0.05

Clinical Chemistry:

There was no treatment-related change in the blood (serum) chemistry.

Urine analysis:

There were no treatment related-changes in the urine chemistry.

Organ weights:

There was no significant change in the organ weight.

Gross Pathology:

There was no treatment-related change in the gross pathology of the treated dogs.

Histopathology:

There was no treatment related histopathology changes observed in the study. Animal that was sacrificed for humane reasons on week 33 (M31869) showed severe multiple abscesses in the abdominal cavity. Lymphocytic infiltration in the conjunctiva (minimal) of the left and right eyes was also observed. The animal showed uterine horn although it was a male dog. Generalized sepsis was reported to be the cause of deterioration of the health condition. The blood samples from the animal were not collected for the determination of the systemic exposure to CSA.

Toxicokinetics for one-year toxicity study in beagle dogs:

Page 124, vol 18:

Blood samples were collected and shipped to Allergan Pharmaceuticals for assay of CSA levels in the blood. The study protocol has been described in page 13. The assay was conducted at Allergan Pharmaceuticals during Oct 96 to Sept 95 and Aug 96 to Sept 96. Blood Cyclosporine levels were determined by validated liquid chromatographic technique.

PK parameters:

C_{max} (ng/ml), C_{min} (ng/ml), Tmax (h) and AUC_{0-T} (ng.hr/ml) were evaluated for week 1 and week 49 of the treatment. The limit of detection was 0.1 ng/ml.

Table L. Cyclosporin A pharmacokinetic parameters determined from the blood concentrations in male and female dogs dosed three times or six times daily with 0.1%, 0.2% and 0.4% cyclosporine emulsions for 49 weeks. Data are reported as Mean ± SD (n), where applicable.

Male Dogs (Week 49)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	5.00 ± 3.46 (3)	0.310 ± 0.051 (3)	BLQ[a]	2.12 ± 0.689 (3)*
0.2%, 3x/day	6.33 ± 1.15 (3)	0.200 ± 0.049 (3)*	BLQ[a]	1.70 ± 0.63 (3)*
0.4%, 6x/day	10.3 ± 2.89 (3)	0.654 ± 0.389 (3)	0.269 ± 0.118 (3)	9.26 ± 6.39 (3)

Female Dogs (Week 49)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	7.00 ± 0 (3)	0.288 ± 0.193 (3)	BLQ[a]	(NC - 3.01)[c]
0.2%, 3x/day	5.00 ± 3.46 (3)	0.717 ± 0.356 (3)	BLQ[a]	5.09 ± 1.83 (3)
0.4%, 6x/day	10.0 ± 2.65 (3)	0.697 ± 0.163 (3)	0.156 ± 0.018 (3)	9.83 ± 1.72 (3)

Male and Female Dogs Combined (Week 49)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	6.00 ± 2.45 (6)	0.299 ± 0.127 (6)*	BLQ[a]	2.35 ± 0.72 (4)*
0.2%, 3x/day	5.67 ± 2.42 (6)	0.459 ± 0.363 (6)	BLQ[a]	3.39 ± 2.22 (6)*
0.4%, 6x/day	10.2 ± 2.48 (6)	0.675 ± 0.268 (6)	0.213 ± 0.098 (6)	9.55 ± 4.19 (6)

* significantly different from that of the 0.4%, 6x/day treatment group ($p < 0.05$)

[a] concentrations were below the limit of quantitation (BLQ) of ——— for ≥ 50% of samples in this group

[b] AUC from time zero to the last detectable concentration at time T ($9 \leq T \leq 24$ hr)

[c] range of AUC_{0-T} values is shown as AUC_{0-T} for 2/3 of the animals was not calculable (NC)

L-1996-4353, pp 322-342

PK-96-023

Table II. Cyclosporin A pharmacokinetic parameters determined from the blood concentrations in male and female dogs dosed three times or six times daily with 0.1%, 0.2% and 0.4% cyclosporine emulsions for one week. Data are reported as Mean ± SD (n), where applicable.

Male Dogs (Week 1)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	6.33 ± 1.15 (3)	0.246 ± 0.163 (3)*	BLQ[a]	(NC - 3.65)[c]
0.2%, 3x/day	4.33 ± 3.06 (3)	0.275 ± 0.031 (3)*	BLQ[a]	2.41 ± 0.18 (3)*
0.4%, 6x/day	8.33 ± 3.06 (3)	0.807 ± 0.356 (3)	0.218 ± 0.201 (3)	13.2 ± 6.4 (3)

Female Dogs (Week 1)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	6.33 ± 1.15 (3)	0.168 ± 0.066 (3)*	BLQ[a]	(NC - 1.64)[c]
0.2%, 3x/day	5.67 ± 1.15 (3)	0.340 ± 0.060 (3)*	BLQ[a]	2.94 ± 0.85 (3)*
0.4%, 6x/day	9.67 ± 2.31 (3)	0.785 ± 0.081 (3)	0.125 ± 0.066 (3)	12.4 ± 1.6 (3)

Male and Female Dogs Combined (Week 1)

Cyclosporine Dose	T_{max} (hour)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-T}[b] (ng.hr/ml)
0.1%, 3x/day	6.33 ± 1.03 (6)	0.207 ± 0.119 (6)*	BLQ[a]	(NC - 3.65)[c]
0.2%, 3x/day	5.00 ± 2.19 (6)	0.307 ± 0.056 (6)*	BLQ[a]	2.67 ± 0.62 (6)*
0.4%, 6x/day	9.00 ± 2.53 (6)	0.796 ± 0.231 (6)	0.172 ± 0.143 (6)	12.8 ± 4.2 (6)

* significantly different from that of the 0.4%, 6x/day treatment group ($p < 0.05$)

[a] concentrations were below the limit of quantitation (BLQ) of ——— for ≥ 50% of samples in this group

[b] AUC from time zero to the last detectable concentration at time T ($11 \leq T \leq 24$ hr)

[c] range of AUC_{0-T} values is shown as AUC_{0-T} for 2/3 of the animals was not calculable (NC)

L-1995-3213, pp 27, 34-44.

Table III. Comparison of trough cyclosporin A concentrations after 1, 12 and 26 weeks of topical ocular dosing of male and female dogs three or six times daily with 0.1%, 0.2% and 0.4% cyclosporine emulsions. Data are reported as Mean ± SD (n), where applicable.

Male Dogs

	Cmin (ng/ml)		
Cyclosporine Dose	Week 1	Week 12	Week 26
0.1%, 3x/day	BLQ[a]	BLQ	BLQ
0.2%, 3x/day	BLQ	BLQ	BLQ
0.4%, 6x/day	0.218 ± 0.201 (3)	0.281 ± 0.082 (3)	0.260 ± 0.080 (3)

Female Dogs

	Cmin (ng/ml)		
Cyclosporine Dose	Week 1	Week 12	Week 26
0.1%, 3x/day	BLQ	BLQ	BLQ
0.2%, 3x/day	BLQ	BLQ	BLQ
0.4%, 6x/day	0.125 ± 0.066 (3)	0.258 ± 0.036 (3)	0.183 ± 0.028 (3)

Male and Female Dogs Combined

	Cmin (ng/ml)		
Cyclosporine Dose	Week 1	Week 12	Week 26
0.1%, 3x/day	BLQ	BLQ	BLQ
0.2%, 3x/day	BLQ	BLQ	BLQ
0.4%, 6x/day	0.172 ± 0.143 (6)	0.270 ± 0.058 (6)	0.222 ± 0.068 (6)

[a] concentration below the limit of quantitation (BLQ) in the group were ≥ 50% of the data point

Summary of the TK study for 52 weeks dog study:

The data suggest that systemic exposure to CSA following topical administration to the eye was increased with the dose. The C_{min} level of CSA after dosing for 49 weeks at 0.4% CSA was 0.2 ng/ml. At lower doses, the C_{min} level was below the limit of quantitation. Dog # 31889 showed maximum blood levels of CSA on weeks 1 (___ ng/ml) and 49 (___ ng/ml). These data suggest that topical applications of CSA ophthalmic emulsion up to 0.4% (One drop 6 times/day) concentration did not show systemic accumulation of CSA. The systemic exposure from the ocular administration was also minimal. The bioavailability of CSA following ophthalmic delivery was minimal.

Key study findings: Lacrimation and redness of the placebo and CSA treated eyes at all CSA doses tested. However, these observations were more pronounced at 0.4% CSA. The clinical formulation contains 0.05% CSA. Therefore, these changes at the clinical dose is expected to be minimal.

Study Title: AGN 192371-Cyclosporine ophthalmic emulsion, A six-month ocular and systemic toxicity study with a two-month recovery period in New Zealand rabbits.

Study number: 1793-2936-6

Amendment: Original NDA, Vol: 15, Page: 001

Conducting Laboratory and Location: Allergan Inc., 2525 Dupont Drive, P.O. Box 19534, Irvine, CA 92713-9534.

Date of study initiation: Sept 14, 1994

GLP Compliance: Yes

Q.A Report: Yes

Dosing:

Species/Strain	#/Sex/Group	Age (months)	Weight (kg)
Rabbits/NZ White, Hra(NZW)SPF	15	4	2.2-2.9 (m); 2.3-3.1 (f)

Satellite group for toxicokinetics:

An additional 3/sex animals were assigned to group 6 (0.4% CSA, 6 times /day). These animals were allotted for the toxicokinetic study. These animals were sacrificed on day 8.

Satellite group for recovery: The first ten surviving animals/sex/gr were sacrificed as scheduled. The remaining animals were allotted to the recovery group. All recovery groups had Five rabbits/sex except for Group 4(CSA 0.2% 3 times/day) which had 4 males and 3 females, and group 6 (CSA 0.4% six times/day), which had 5 males and 4 females.

Dosage groups in administered units:

Group	No of animals and sex	Test Formulation	Frequency of dosing
1	15 M, 15 F	Vehicle of CSA 0.2%	3 times/day
2	15 M, 15 F	Vehicle of CSA 0.4%	6 times/day
3	15 M, 15 F	CSA 0.05%	3 times /day
4	15 M, 15F	CSA 0.2%	3 times/day
5	15 M, 15F	CSA 0.4%	3 times/day
6	15 M, 15F	CSA 0.4%	6 times/day

Route, form and volume:

CSA ophthalmic emulsions or vehicles were applied as drops. Each drop was considered to be 40 µL. Drops of CSA emulsion or the vehicle were instilled into the lower conjunctival sac of the left eye for six months according to the daily schedule as shown in the table above. The right eye served as the untreated control.

Intervals between the doses for TID administration were 3 hours. The intervals between the doses for the 6X/day administration were 2 hours.

Animals in the groups 1, 3, 4 and 5 were dosed one drop BID at 3 hour intervals on the day of the eye examination. Animals in groups 2 and 6 were given one drop QID at 1-3 hour intervals on the day of eye examinations.

Drug Lot # and purity:

Following test and control articles were used:

1. Vehicle of Cyclosporine 0.2% ophthalmic emulsion, Lot # 8747X-10651
2. Vehicle of Cyclosporine 0.4% ophthalmic emulsion, Lot # 8746X-10619A and 10619B
3. Cyclosporine 0.05% ophthalmic emulsion, Lot # 8736X-10650B (actual concentration was 0.047%)
4. Cyclosporine 0.2% ophthalmic emulsion, Lot # 8734X-10621A (actual concentration was 0.189%)
5. Cyclosporine 0.4% ophthalmic emulsion, Lot # 8733X-10622A (actual concentration was 0.39% and 10622B (actual concentration was 0.401%)

Actual concentrations were determined by the analytical testing.

The formulation was preservative free. Compositions of the formulations of the dosage form or the placebo were not given in the report. However, the compositions of the lots used in the study are attached in this review from the chemistry review. The pH of the formulations was close to 7.4.

Observations and times:

Clinical signs:

Mortality was checked daily. Clinical signs for side effects and toxicity were observed daily. Gross ocular examinations were made after the first dose and last dose daily during the first week of the treatment. The gross ocular examinations were made on a weekly basis from the second week. In addition, a pre-instillation gross ocular examination was performed before the first and last treatment on days 2, 6 and once weekly for rest of the study for the determination of the ocular irritancy and discomfort.

Gross ocular examinations were performed once weekly during the recovery period.

Body weight: Individual body weights were recorded prior to the beginning of the study and weekly thereafter. Body weights of the animals were also recorded on the day of necropsy.

Food consumption: The sponsor has not mentioned in the study report when the food consumption data were recorded.

Ocular examination:

Both eyes were examined by an ophthalmoscope at screening and randomization days -13, -12, -7, at one month, three months, at the end of the treatment period and recovery period. Effect of the treatment on lens, vitreous and retina were examined among other ocular tissues. As stated in the methods, ocular status of each animal was also examined by slit lamp biomicroscope on days-6, -5 and -4, at the end of one month, end of the treatment and recovery periods. The protocol and the results have not specified whether one or three examinations were conducted at the base line. However, results showed only one set of data at the baseline.

EKG: Not recorded.

Hematology:

About 4 ml blood samples were taken from the over night fasted animals on days -6, -5, -4, end of three months, end of the treatment and recovery periods. Samples were used for hematology and blood chemistry examinations. Number of samples taken at the baseline is not mentioned. However, results showed one set of data at the baseline.

Following parameters were determined.

Hematocrit, red blood cell count, hemoglobin, white cell count, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, absolute and differential WBC counts.

Blood chemistry tests:

AST, ALT, albumin, albumin/globulin ratio, AP, bilirubin, blood urea nitrogen, BUN/creatinine ratio, calcium, chloride, cholesterol, creatinine,

Drug Blood Levels:

Plasma samples for toxicokinetics were taken on day 7 and during week 8 from the satellite animals and/or main study animals. Plasma as well as blood samples were taken during last week of the study from the main study animals. Times of sampling after the first dose were 0 and 2 hours for groups one and two; 0, 0.5, 2, 3.5, 5, 6.5, 8, 12 and 24 hours for the groups 3, 4 and 5; and 0, 0.5, 3.0, 5.0, 6.5, 9.0, 10.5, 12 and 24 hours for group 6.

Urine analysis:

Not performed.

Gross Pathology:

Animals were euthanized with pentobarbital at the end of the experiment and necropsy was performed. Gross pathological changes were examined for major organs.

Organ weights:

Following tissues and organs were weighed.

Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen and testes.

Histopathology:

Following tissues and organs were preserved in 10% buffered formalin. However, histological examinations were conducted in all tissues for group 1 and 6 only. Any tissue or organ that showed drug related microscopic changes was further examined for all other groups.

Ocular tissues from all groups were examined for histological changes.

Adrenal glands, aorta, bone marrow (femur), brain, cervix, diaphragm, epididymides, eyes with optic nerves and surrounding structures, gall bladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, heart, kidneys, liver, lungs, cervical lymph nodes, mediastinal lymph nodes, mesenteric lymph nodes, mammary gland, ovaries, pancreas, pituitary gland, prostate gland, salivary glands (including parotid and sub-maxillary), sciatic nerve, seminal vesicle, skeletal muscle, spinalcord, spleen, testes, sternum, thymus, thyroid glands with parathyroids, tongue, trachea, urinary bladder, uterus and vagina.

Results:

Mortality and clinical signs:

There was no treatment-related mortality. However, following animals were sacrificed before termination of the experiment due to humane reasons.

Group	Animal	Day	Remarks
4	F #7040	8	Broken back during bleeding procedure
4	M # 6959	91	Broken back during bleeding procedure
4	F #7075	139	Buphthalmia, increased IOP, Iritis in both eyes[1]
6	F #7046	8	abnormal neurological signs[2]

[1] There was no histological abnormality in the eye. Cloudiness of the cornea was observed in right eye on day 112 and on left eye on day 113. The condition was considered to be unrelated to the treatment because it started in the untreated eye. Also there was no histological abnormality in the eye.

[2] fixed pupil, rigid stance, clamped jaws and dehydration were observed. Nasal swab showed Staphylococcus infection.

The condition of above listed animals was not considered to be treatment related. There was no other clinical conditions reported that could be related to the treatment.

Gross ocular findings are shown in the following tables.

Table 1
Total Incidence of Gross Ocular Findings - Post Daily First and Last Instillation (Male)

GROUP	TEST ARTICLE		DISCOMFORT b		HYPEREMIA c	
		Instillations scored[a]	Score	Incidence (%)	Score	Incidence (%)
1 (3X/day)	Vehicle of 0.2% Cyclosporine	940	1,1 1,2	382 (41%) 1 (<1%)	1	78 (8%)
2 (6X/day)	Vehicle of 0.4% Cyclosporine	940	1,1	517 (55%)	1	204 (22%)
3 (3X/day)	Cyclosporine 0.05%	940	1,1 1,2	522 (55%) 4 (<1%)	1 2	284 (30%) 3 (<1%)
4 (3X/day)	Cyclosporine 0.2%	914	1,1 1,2	662 (72%) 1 (<1%)	1 2	236 (26%) 3 (<1%)
5 (3X/day)	Cyclosporine 0.4%	940	1,1	650 (69%)	1	343 (36%)
6 (6X/day)	Cyclosporine 0.4%	940	1,1 1,2	549 (58%) 6 (<1%)	+1 +2	314 (33%) 4 (<1%)

Total Incidence of Gross Ocular Findings - Post Daily First and Last Instillation (Female)

GROUP	TEST ARTICLE		DISCOMFORT b		HYPEREMIA c	
		Instillations scored[a]	Score	Incidence (%)	Score	Incidence (%)
1 (3X/day)	Vehicle of 0.2% Cyclosporine	940	1,1 1,2	384 (41%) 2 (<1%)	1	55 (6%)
2 (6X/day)	Vehicle of 0.4% Cyclosporine	940	1,1	523 (56%)	1	211 (22%)
3 (3X/day)	Cyclosporine 0.05%	940	1,1	491 (52%)	1	159 (17%)
4 (3X/day)	Cyclosporine 0.2%	876	1,1 1,2	610 (70%) 1 (<1%)	1 2	170 (19%) 1 (<1%)
5 (3X/day)	Cyclosporine 0.4%	940	1,1 1,2	679(72%) 1 (<1%)	1 2	263 (28%) 1 (<1%)
6 (6X/day)	Cyclosporine 0.4%	889	1,1 1,2	552 (62%) 3 (<1%)	1	282 (32%)

a The first and last instillation were scored daily during the first week and once weekly for the remainder of the study. The scores presented are from the treatment period.

b Discomfort: severity score: (1 = slight; 2 = mild; 3 = moderate; 4 = severe)
duration score: (1 = 1-30 seconds; 2 = 30 seconds-1 minute; 3 = 1-2 minutes; 4 = 2-5 minutes)

c Hyperemia: severity score: (1 = slight; 2 = moderate; 3 = severe)

Table 2
Total Incidence of Gross Ocular Findings - Pre Daily First and Last Instillation (Male)

Group	TEST ARTICLE	HYPEREMIA b		
		Instillations scored[a]	Score	Incidence (%)
1 (3X/day)	Vehicle of 0.2% Cyclosporine	780	1 *	8 (1%) 1 (<1%)
2 (6X/day)	Vehicle of 0.4% Cyclosporine	780	1	11 (1%)
3 (3X/day)	Cyclosporine 0.05%	780	1 *	48 (6%) 1 (<1%)
4 (3X/day)	Cyclosporine 0.2%	756	1	23 (3%)
5 (3X/day)	Cyclosporine 0.4%	780	1	46 (6%)
6 (6X/day)	Cyclosporine 0.4%	780	1 2	44 (6%) 1 (<1%)

Total Incidence of Gross Ocular Findings - Pre Daily First and Last Instillation (Female)

Group	TEST ARTICLE	HYPEREMIA b		
		Instillations scored[a]	Score	Incidence (%)
1 (3X/day)	Vehicle of 0.2% Cyclosporine	780	1	3 (<1%)
2 (6X/day)	Vehicle of 0.4% Cyclosporine	780	1	10 (1%)
3 (3X/day)	Cyclosporine 0.05%	780	1	17 (2%)
4 (3X/day)	Cyclosporine 0.2%	720	1	13 (2%)
5 (3X/day)	Cyclosporine 0.4%	780	1	28 (4%)
6 (6X/day)	Cyclosporine 0.4%	732	1	22 (3%)

* = Tearing

a Pre-instillation scores were obtained weekly. Scores from the treatment period are presented.

b Hyperemia: severity score: (1 = slight; 2 = moderate; 3 = severe)

Following instillation, slight to mild ocular discomfort was observed for 30 to 60 sec in the vehicle and CSA treated animals. Similarly, slight conjunctival hyperemia was also observed in the vehicle and CSA treated animals. Ocular discomfort and conjunctival hyperemia could be related to the vehicle.

Prior to first and last daily instillation, slight hyperemia was observed in the vehicle and CSA treated animals. However, CSA treated eyes showed higher incidences of hyperemia.

The sponsor stated that miosis primarily in conjunction with iritis were seen during the first week at 0.4% 6x/day dose in 5 male and 5 female animals as *gross ocular observations*. Two males also showed miosis at 0.05%CSA. There were incidences of tearing in female animals in groups 1,3 and 4.

Body weights:

Body weight was not affected by the treatment. The average body weight (kg) is shown in the following table.

	Gr1,veh .2%		Gr2,veh .4%		Gr3,.05%		Gr4, .25%		Gr5,.4%		Gr6, .4%6x	
	M	F	M	F	M	F	M	F	M	F	M	F
Wk 0	2.57	2.65	2.57	2.65	2.57	2.65	2.57	2.65	2.57	2.66	2.57	2.66
Wk 26	3.12	3.52	3.08	3.48	3.16	3.47	3.20	3.49	3.12	3.48	3.04	3.49
Wt gain	.55	.87	.51	.83	.59	.82	.63	.84	.55	.82	.47	.83

Food consumption:

No data provided in the report

Ophthalmoscopy and slit lamp examinations:

The findings of animal # 7075 (gr 4 Female) already shown in the result.

Ophthalmoscopic examinations did not show abnormalities in lens, vitreous or retina at the end of one, three and six months. Animal # 7075, 0.2%, F, showed cloudiness in the cornea on day 112.

Slit lamp examinations showed slight-mild iritis in 7 animals (4M,3F) at 0.4% six-time dosing during first week of the study. Isolated incidences of iritis were also noted in two animals at 0.05% CSA (gr 3) and one animal at 0.4% CSA (gr 5) during the first week.

Slight to moderate conjunctival congestion and slight discharge were observed at 0.4%, 6x Cyclosporine ophthalmic solution during one to six month of observation. Conjunctival congestion was observed at 0.05 and 0.2%, 0.4% 3x and the vehicle of 0.4% CSA. Conjunctival discharge was observed at 0.2% CSA at three months. However, number of incidences was limited (see page 30).

The report mentioned that there were incidental findings of opacity (# 6910, # 7132 0.05% CSA; vehicle for 0.4% CSA # 6974) observed during the study. However, these incidences were considered to be incidental. Data for the slit lamp examinations for one, three and six months are shown in the following tables.

Table 3
Slit Lamp Biomicroscopy: Scheduled Examinations
Findings in the Left (Treated) Eye

Number of Animals Affected (% Incidence)

Time	Group	Treatment	Sex	Congestion[a]		Discharge[b]	Other
				1	2	1	
One Month	2	Vehicle of 0.4%	M	3 (20)	0	0	0
	3	0.05% cyclosporine	F	0	0	0	†
	5	0.4% cyclosporine 3X/day	M	1 (7)	0	0	0
	6	0.4% cyclosporine 6X/day	M	9 (60)	3(20)	2 (13)	0
			F	5 (36)	0	3 (21)	0
Three Months	2	Vehicle of 0.4%	M	1 (7)	0	0	*
	3	0.05% cyclosporine	M	1 (7)	0	0	0
			F	0	0	0	†
	4	0.2% cyclosporine	F	1 (7)	0	1 (7)	0
	5	0.4% cyclosporine 3X/day	M	2 (13)	0	0	0
			F	1 (7)	0	0	0
	6	0.4% cyclosporine 6X/day	M	4 (27)	1	2 (13)	0
			F	4 (27)	0	2 (14)	0
Six Months	3	0.05% cyclosporine	M	1 (7)	0	0	0
			F	0	0	0	†
	5	0.4% cyclosporine 3X/day	M	1 (7)	0	0	0
	6	0.4% cyclosporine 6X/day	M	2 (13)	0	2 (13)	0

* One animal with a slight (+1) corneal opacity involving less than 25% of the left eye with no associated fluorescein staining.

† One animal with a slight (+1) scratch-like corneal opacity involving less than 25% of the left eye with no associated fluorescein staining.

[a] Scoring for congestion of the conjunctiva: 1 = slight, 2 = moderate, 3 = severe

[b] Scoring for discharge: 1= slight, 2 = moderate, 3 = severe

Table 4
Slit Lamp Biomicroscopy: Unscheduled Examinations
Findings in the Left (Treated) Eye of Males and Females

Number of Animals Affected

Time	Group & Treatment	Congestion[a]		Flare[b]	Iritis[c]		other
		1	2	1	1	2	
Day 2	Group 6, 6X/day 0.4% cyclosporine	0	0	0	6	0	0
Day 2	Group 5 0.4% cyclosporine	0	0	0	1	0	@
Day 3	Group 6, 6X/day 0.4% cyclosporine	0	1	1	0	1	0
Day 4	Group 6, 6X/day 0.4% cyclosporine	1	0	0	1	0	0
Day 6	Group 3 0.05% cyclosporine	1	0	1	1	0	0
Day 7	Group 3 0.05% cyclosporine	1	0	1	1	0	*
Day 77	Group 6 6X/day 0.4% cyclosporine	0	1	0	0	0	†

@ One animal had slight (+1) discharge in the left eye.

* One animal had a mild (+2) scratch-like corneal opacity with associated moderate fluorescein staining (+1) affecting less than 25% of the cornea, and slight (+1) discharge in the left eye.

† The same animal had slight (+1) congestion in the right eye and slight (+1) discharge in the left eye.

This table does not include data for group 4 female no. 7075 which can be found in appendix VI and section IV of this report.

[a] Congestion of the conjunctiva: 1 = slight, 2 = moderate, 3 = severe
[b] Aqueous Flare: 1= slight, 2 = moderate, 3 = severe
[c] Iritis: 1 = slight, 2 = mild, 3 = moderate

Hematology

Data did not show any treatment-related change in male and female animals. However, female animals showed statistically significant changes in the hematocrit (grs 2,3,4,5) and platelets counts (grs 5,6), differential counts for neutrophil and lymphocyte (gr 3) at the end of three months. Except platelet counts, there was no statistically significant change that was observed at the end of six month in female rabbits. Group 6 female # 7083 had platelet counts of ——— and # 7125 had platelet counts of ——— on pretest, month three and six, respectively. Platelet counts for other female rabbits on 3 and 6 months of observation were within the range of pretest values. Therefore, hematological changes were considered to be incidental. The data are shown in the following table.

Gr, Wk	HCT (%)	PLT, 10^3	Neut (%)	Lymp (%), WBC $10^3/mm^3$	Wk	Platelet
1, 13	38.8	289	14.5	78.1, 7.52	26	273
2, 13	36.6*	280	16.7	75.2, 7.15	26	288
3, 13	37*	306	17.8*	74.0*, 7.13	26	296
4, 13	36.3*	298	14.9	76.6, 8.03	26	297
5, 13	36.6*	352*	13.7	78.7, 7.97	26	307
6, 13	37	341*	13.7	78.9, 7.81	26	338*

*Significantly different from respective the control for the treated group. The change in group 2 was compared to group 1.

Clinical Chemistry:

Changes during the treatment period in male and female animals are shown in the following table.

Male

Gr, Wk	Pr g/dl	Ind.Bili mg/dl	Wk	Pr, g/dl	Phos mg/dl	Glob g/dl	Al/Gl
1, 13	5.6	0.4	26	5.5	4.8	1.1	4.0
2, 13	5.6	0.4	26	5.3	5.3	0.9*	5.2*
3, 13	5.8	0.5*	26	5.3	5.4*	1.0*	4.6
4, 13	5.6	0.4	26	5.2*	4.5	0.9*	4.8*
5, 13	5.9*	0.5*	26	5.5	4.9	1.1	4.1
6, 13	5.9*	0.5*	26	5.6*	5.4	1.2*	3.9*

Female (week 13)

Group	Calcium mg/dl	Potassium (g/dl)	Phos mg/dl	LDL IU/l	BUN/Creat	Globulin g/dl
1	13.1	5.4	4.9	48	15	3.6
2	12.8*	5.2*	5.0	51	16	4.1*
3	12.9	5.4	5.1	53	17*	3.7
4	12.8*	5.3	5.4	41	17*	4.1*
5	12.9	5.5	5.6	96*	18*	4.0*
6	13.2	5.5	5.2	84*	16	3.8

Female (week 26)

Group	Pot, mmol/l	Tot Bili, mg/dl	Indirect Bilirubin, mg/dl
1	3.8	0.6	0.6
2	3.9	0.5	0.5
3	3.8	0.6	0.6
4	4.3*	0.6	0.6
5	3.8	0.6	0.6
6	3.7	0.6*	0.6*

* Statistically significant from the respective control. The change in group 2 was compared with group 1.

Above changes were considered to be incidental due to smaller magnitude. Blood chemistry data did not show trend of any abnormality. Overall CSA ophthalmic emulsion did not show any systemic changes for the laboratory parameters.

Urine analysis:

Not conducted.

Organ Weights:

Absolute and relative organ weight data showed a decrease in the weight of spleen in male animals at 0.4% six-times/day dose compared to the vehicle. The data (mean +/- SD, n=10) are shown in the following table.

Group	Wt. of spleen (g)	Wt of spleen (g, % of BW)
Group 2, 0.4% vehicle x6	1.0434 + 0.25468	0.0339 + 0.00824
Group 6, 0.4% CSA x6	0.7632* + 0.24217	0.0248* + 0.00676

* $P<0.05$

Animal #6926 and #6954 in group 6 showed lower weight of spleen compared to individual animals in the group 2. Data for these two animals in the group 6 could contribute to the decrease in the average weight of spleen. The maximum blood levels of CSA and the weight of spleen were compared among some of the group 6 male animals as shown in the table below.

Animals	Spleen wt (g)	Maximum blood levels (ng/ml)
6901		
6926		
6932		not available
6944		
6954		not available
6949		not available
6950		not available
6955		not available
6976		not available
6983		not available

The reduction of the weight of spleen could be due to the increase in blood levels of CSA. However, in the absence of a similar relationship between the weight of the spleen and blood levels of CSA among female rabbits, the effect of CSA treatment on the weight of spleen was considered to be incidental. Data for the weight of the spleen and CSA levels for group 6 female rabbits are shown in the following table.

Animals	Spleen weight (g)	Maximum blood levels (ng/ml)
7022		
7035		
7114		
7050		
7061		not available
7073		not available
7083		not available
7099		not available
7105		not available
7113		not available

Gross pathology:

There was no treatment related gross pathological changes reported in the report.

Histopathology:

There was no CSA related histological changes in the non-ocular tissues.

Histological changes in the ocular tissues of male rabbits are shown in the following table.

Lesions	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6
Cornea (L), mononuclear cell, min	0	1	1	1	0	2
Upper eyelid (L), mononuclear, min	6	3	5	5	2	6
Dermis, minimal	3	4	1	2	2	2
Dermis, mild	0	2	0	1	0	0
Nic. membrane, mononuclear, min	5	2	1	1	3	5
Nic. gland, mononuclear. cell, min	0	1	0	2	2	2
Lower eyelid (L), mononuclear. min	7	6	8	5	9	5
mild	2	0	1	3	1	2
mod	0	0	1	0	0	0
Inflamm. cell infilt. dermis, min	5	4	0	2	2	1
mild	0	0	1	0	0	0
Lacrimal gl, (L), mononucl cell, min	0	0	0	0	1	0
Cornea (R), mononucl. cell, min	0	0	0	1	0	0
Upper eyelid (R), mononucl, min	3	5	7	8	3	2
Upper eyelid (R), mononucl.mod	1	0	0	0	0	0
mononucl, dermis, min	1	2	0	1	0	0
Nic mem R, mononuclear. cell, min	5	2	1	2	4	3
Nic gl., min	0	1	1	0	1	1
Lower eyelid R, mononucl cell, min	8	6	8	5	8	7
mild	0	0	1	3	2	1
mod	0	0	0	0	0	1
mononucl. cell infiltr. dermis,min	4	3	1	2	3	0
Lacrimal gland R, mononucl cell, min	0	1	1	0	0	0
Harder's Gl (L),Chronic Inflamm, min	2	0	1	0	0	0
mild	0	1	0	1	1	1
Harder's. Gland. Mononucl.cell, min	1	0	1	1	1	1
Harder's Gland R,Chronic inflam, min	1	1	2	0	0	2
mild	0	0	1	1	1	0
mod	0	0	1	0	0	0
Mononuclear. cell infiltration, min	0	1	0	0	0	0

R = Right eye, L= Left eye, Min= minimal, Mod= moderate, Inflam = Inflammation, Gl = Gland, Nic = Nictitating membrane, mononucl = Mononuclear, Infil = Infiltration, Ch = Chronic. Number of animals examined per group and sex = 10. Similar abbreviations are used for the female animals.

Histological changes in the ocular tissues in female rabbits are shown in the following table.

Lesions	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6
Cornea (L), mononuclear cell, min	0	1	0	0	0	0
Upper eyelid (L), mononuclear min	4	9	7	6	7	6
Upper eyelid (L), dermis, min	5	1	3	0	5	3
mild	0	1	0	0	1	3
Nic. membrane, mononuclear., min	3	1	4	3	3	3
Nic. gland, mononuclear cell, min	0	0	0	0	0	1
Lower eyelid (L),mononuclear, min	7	7	8	9	7	6
mild	3	2	1	1	3	0
Inflamm. cell infiltration. dermis, min	5	2	2	3	4	1
mild	0	1	0	0	1	2
mod	0	1	0	0	0	0
Dermatitis, mild	0	1	0	0	0	0
Lacrimal gl (L), mononucl. cell, min	5	1	0	1	2	2
Cornea R, mononucl. cell, min	0	1	0	0	1	0
Upper eyelid R,mononuclear cell, min	5	5	5	5	7	8
Mononucl cell, dermis, min	2	4	1	4	5	3
Nic. membrane R, mononuclear, min	2	4	2	3	2	1
mild	0	0	1	1	0	0
Nic gland, min	0	0	1	1	0	1
Lower eyelid R,mononuclear cell, min	5	9	7	7	8	7
mild	4	0	1	3	2	1
mod	1	0	0	0	0	0
Mononuclear cell infiltr.dermis, min	3	2	4	6	9	4
mild	1	0	0	0	0	1
Lacrimal gland R, mononuclear, min	3	2	2	3	2	0
Harder's Gl (L),Chronic.Inflamm, min	0	1	0	1	1	0
mild	0	0	1	0	0	0
Mononucl. cell infiltration., min	0	0	0	1	1	0
Harder's gl R,Ch Inflammation, min	0	0	1	1	1	0

Histology data show inflammatory cells in the upper, lower eyelids and submucosal layer, lacrimal and Harder's glands in the left and right eyes in placebo and CSA treated animals. There were similar changes in the untreated right eyes and treated left eyes. Data suggest that the CSA and placebo treatment showed no toxic effects in the animals. Presence of inflammatory cells in the right and left eyes may be spontaneous. The reviewer suggests that a separate group of untreated animals should have been included in the study for a comparison.

There is no sign of systemic toxicity based on the histology data from non-ocular tissues. No histological abnormalities were observed in the anterior chamber, iris, ciliary body, lens, retina, sclera, choroid, optic nerve and extraocular muscles.

Toxicokinetics:

Whole blood levels of CSA at the end of the treatment are shown in the following table. The sponsor analyzed the whole blood levels (n=6-8) although plasma samples were also taken for the assay. The sponsor reasoned that whole blood levels were higher that helped them to quantitate the level reliably. Following statement was made by the sponsor in page 021, vol 15, "Plasma samples for toxicokinetic determinations were taken on day seven and during week eight from the satellite animals and/or main study animals, and plasma as well as blood samples for toxicokinetic analysis were taken during the last week of the study from the main study animals. Since the data in the literature showed that the concentrations of Cyclosporine are higher in blood than in plasma, it was decided to analyze only the drug concentrations in whole blood which were taken at the end of the study". Data summarized for male and female rabbits.

CSA Dose	T_{max} (h)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-24} (ng.hr/ml)
0.05%	6.21	0.328	below detec	3.48
0.2%	6.06	0.997	below detec	9.25
0.4%	5.69	0.570	below detec	6.85
0.4% 6X/day	6.00	1.36	.29	16.7

The highest detected blood levels for male rabbit # 6901, 6926, 6932, 6944 were ng/ml, respectively. The highest detected blood levels for female # 7022, 7035, 7114, and 7050 were and ng/ml, respectively. The highest blood CSA levels (ng/ml) were detected in male # 6921 at 12 hours at 0.2% CSA. Blood exposure to CSA increased with doses at the end of six months and highest average exposure was noted at 0.4% 6 times dosing. The data suggest that Cyclosporine ophthalmic doses were bioavailable to the systemic circulation. However, bioavailability was minimal.

Key study findings:

CSA ophthalmic emulsion or placebo could induce conjunctival hyperemia (slight), conjunctival discharge (slight) and discomfort (slight-mild). Slight to mild iritis was noted at 0.4% 6X CSA dose during first week of the treatment. Miosis was observed at 0.4% 6X CSA during first week of the dosing. No other toxicity related to CSA was observed in the eye or non-ocular tissues.

Special Technical Report:

Cyclosporine A: [redacted] special study.

Study # TR: PA-1998-010:

Vol 20, Page 002

Location: Allergan Spectroscopy Lab, Irvine, CA and [redacted]

Date of study Initiation: Not mentioned

GLP compliance: No

QA Reports: No

Methods: Unknown [redacted] substance was quantitated from the [redacted] using [redacted]. The study was part of a chemical stability protocol. The reviewing chemist will review the report. The [redacted] impurity was identified from the [redacted] from clinical batches for the Phase III trials.

Lot #:

The [redacted] impurity was detected from following batches:

11101, 11102, 11108, 11109, 11138, 11139, and 11143. These batches were from the clinical study. Freshly manufactured lot # 11234, 11142 and 11235 were also used for the purpose of validation of the method.

Results:

The [redacted] was identified as [redacted] molecular weight [redacted] The [redacted] impurity was [redacted]

The data for the freshly prepared CSA emulsion for the presence of [redacted] is shown below. The impurity was [redacted]

Sample, lot #	ppm
11234	[redacted]
11142	[redacted]
11235	[redacted]

replicated similar test. The data from ranged from ppm. The clinical batches were examined for the presence of . using '

A correction factor of was applied for the use of The amount of . in the clinical batches is shown below.

Sublot	Concentration ppm
11101A	
11102A	
11108A	
11109A	
11138A	
11139A	
11143A	

The maximum concentration of was ' ppm or

Summary:

Clinical batches of CSA showed an impurity identified as The maximum amount detected was about ppm.

The concentration of CSA in the formulation is 0.05%. Considering 35 µL as the volume per drop, each drop of the formulation contains 17.5 µg of CSA. The maximum concentration of the impurity is Each drop would contain of . The sponsor submitted several published reports that suggest is present in

The amount of in each drop is considerably lower than that found in the Therefore, possibility of any systemic adverse effect from the ophthalmic formulation is limited. The clinical batches used for the Phase III trial also had similar impurity. If the impurity contributes to ocular toxicity that would have been detected in the clinical safety report.

Key findings:

The stability data showed an impurity from the The impurity is identified as The maximum amount is ppm or Each 35 µL eye drop will contain of

Overall Toxicology Summary:

Ocular and systemic toxicity to 0.1%, 0.2% and 0.4% Cyclosporine ophthalmic emulsions were examined in beagle dogs for 52 weeks. Control animals received the vehicle for 0.4% CSA. Eye drops were instilled in the left eye and the right eye served as the untreated control. The treatment showed lacrimation to the treated eye due to placebo and CSA emulsions. However, 0.4% CSA showed most lacrimation. Redness to conjunctiva was also observed in the treated eye at 0.1- 0.4%. Although placebo treated animals showed redness, the prevalence was low. There was no other treatment-related toxicity observed in the eye or in other organs examined. The blood pharmacokinetic data showed minimal systemic exposure to CSA. CSA did not accumulate in the blood. Literature data suggest that the IC_{50} for mixed lymphocyte reaction (MLR) for CSA is 40 ng/ml in dogs in vitro. The maximum level of CSA in the blood was [redacted] ng/ml in dogs. These data suggest that ophthalmic doses of CSA would not show systemic immunosuppression.

In another study, Cyclosporine ophthalmic emulsions were instilled into the left eye for six months in male and female rabbits at 0.05%, 0.2% and 0.4% concentrations. Control animals received vehicle only. The contralateral eye served as the untreated control. At 0.4% 6x/day dose, iritis (slight-mild) and miosis were observed in the treated eye (during the first week of treatment). Conjunctival discomfort (slight-mild) and conjunctival hyperemia (slight) were also observed in the placebo and CSA treated rabbits. Slight conjunctival discharge was also observed in CSA treated animals. Inflammation in both eyes in the drug and placebo treated animals with mononuclear cell infiltration was observed in the upper, lower eyelids, lacrimal and Harder's glands. No histological abnormality due to CSA emulsion was noted in the iris, ciliary body, lens, retina, sclera, choroid, optic nerve, extra-ocular muscle and other non-ocular tissues. Systemic levels (C_{max}) of CSA at 0.4% CSA emulsion at a dose of one drop 6 times per day the drug in the whole blood was [redacted] ng/ml.

The systemic trough level in whole blood in rheumatoid arthritic patients is 97 ng/ml. Data suggest that CSA systemic exposure was minimal in dogs and rabbits compared to oral doses in RA patients following ophthalmic delivery of CSA emulsions. There is no evidence of CSA related toxicity to eyes and other organs following the chronic treatment with the CSA ophthalmic formulation.

Stability study in clinical batches of the formulation showed the presence of [redacted] as an [redacted] impurity from the [redacted] The maximum amount detected was about [redacted] Considering the [redacted] and small amount / [redacted] of the impurity in each drop, presence of [redacted] does not have toxicity concern in the eye. The estimated daily exposure to both eyes would be [redacted] Estimated CSA daily exposure to eyes would be about [redacted] which is about 8 fold higher than [redacted] . Also, any untoward reactions to the [redacted] impurity would have been revealed during the clinical trial since the clinical batch also had the [redacted] impurity. The sponsor stated that the preclinical batches did not show [redacted] The sources of the [redacted] used in the preclinical [redacted] were [redacted] The [redacted] for clinical batches were made from [redacted] The sponsor provided literature

suggesting that [redacted] is also present in the [redacted] There is no guideline concerning the limit for [redacted] impurities of the [redacted] used in the antibiotics and biotechnology products. Guidelines (ICH) exist for the threshold of impurities e.g degradation products, reaction products of active ingredients and container/closure system for the synthetic drug products. The acceptable limit of impurities of synthetic drug products in the ICH guideline is 1% or 5 µg total daily dose whichever is lower. The limit of impurities is recommended for the drug product that is used at a maximum daily dose of 1 mg or less. If this guideline is applied to CSA ophthalmic suspensions, the toxicity to [redacted] needs to be characterized. The issue was discussed with the medical reviewer during IND review process. However, it was felt that additional preclinical toxicity data on [redacted] might not be necessary since the clinical data would be sufficient to address the issue.

Pharmacokinetics and ADME studies:

Clinical studies:

Multicenter, double blind, randomized, vehicle controlled safety and efficacy of Cyclosporine 0.05% and 0.1% ophthalmic emulsions used twice daily up to one year in patients with moderate to severe keratoconjunctivitis sicca.

Study # 192371-002, page 240, vol 20

Study dates: July 1997 to June 1998

The pharmacokinetic data from the study would be presented here for comparison to the animal kinetics data if necessary.

CSA blood trough levels, blood CSA area under the curve, tear CSA area under the curve were determined. The samples were assayed by [redacted] method at 0.1ng/ml limit of detection.

The trough blood concentrations of CSA from 0.05% CSA was below the limit of detection. The trough levels in the blood were quantified in six samples at 0.1% dose. These data are shown below.

Month	Trough Levels in blood, ng/ml
1	[redacted]
6	[redacted]

The mean blood trough levels of CSA at the end of six months were below the limit of detection at 0.05% and 0.1% CSA. It was concluded that CSA did not accumulate in the blood after multiple ophthalmic doses. The sponsor indicated that the systemic blood trough levels at 5mg/kg oral dose of CSA was [redacted] ng/ml, Cmax 728 ng/ml.

Data suggest that the systemic exposure to CSA following ophthalmic delivery of CSA (0.05-0.1%) at one drop twice daily dose was minimal compared to the systemic exposure from an oral dose of 5 mg/kg in RA patients.

Ocular distribution and absorption:

Ocular PK of 0.2% ^{3}H-CSA after a single eye drop into rabbit eyes.

Page 257, vol 18, PK-95-010:

A single dose of 50 µL 0.2% ^{3}H-CSA ophthalmic emulsion was instilled into the left eye. The contralateral eye served as the control. The ophthalmic formulation is shown in the following table.

Cyclosporine USP and ^{3}H-CSA	0.2% w/w
Castore oil	
Polysorbate 80	
Pemulen	
Glycerine	
NaOH	
Purified water	
pH	

The lot number of the emulsion was 10678. The study was conducted according to the GLP.

46 female and 14 male NZ rabbits were used in the study. Body weights of the animals were between 2-4 Kg. The One drop of the 0.2% CSA ophthalmic emulsion was instilled into the left eye to groups of 4 female per time point. Animals were sacrificed at 20, 40 min, 1, 2, 4, 6, 12, 24, 48 and 96 hours after dosing for tissue collection. Groups of 4 male rabbits per time point were sacrificed at 40 min, 2 and 24 hours after dosing for tissue collection.

Radioactivity was analyzed from the following tissues:

Tears, nictitating membrane, lower bulbar conjunctiva, upper bulbar conjunctiva, cornea, sclera, aqueous humor, iris-ciliary body, lens, vitreous humor, choroid-retina, optic nerve head, lacrimal gland, blood and plasma.

Tear and blood samples were collected before the sacrifice. Immediately after sacrifice, aqueous humor and other ocular tissues and fluids were collected.

Results:

Radiochemical and chemical purity of the test substance was about 98%. The stability of Cyclosporine in the emulsion was confirmed. The predose and post dose CSA concentration was about

The CSA level as measured from the radioactivity in tears was reduced from 279000 ng eq/ml at about 20 mins after the dosing to 5060 ng eq/ml within one hour after the instillation of ^{3}H-CSA. The level was further reduced to 648 ng eq/ml at 12 hr. Thereafter, it was reduced to about 21.7 ng eq/ml at 96 hours.

The CSA level in the nictitating membrane at 20 mins after the ^{3}H-CSA was 1540 ng eq/g. At the end of 1 hour, about 1200 ng eq/g of Cyclosporine (as radioactivity) was observed in the nictitating membrane. The CSA level in the nictitating membrane was reduced to 228 ng eq/g at the end of 12 hours. The

concentration of radioactivity in the nictitating membrane at the end of 96 hours was about 5.35 ng-eq/g.

The CSA level in upper and lower conjunctiva was 836 and 753 ng eq/g, respectively, at about 20 mins after the administration of ^{3}H-CSA in female rabbits. The level was reduced to 178 and 111 ng eq/g for upper and lower conjunctiva, respectively. The level of CSA in the upper and lower conjunctiva was further reduced to 4.93 and 4.35 ng eq/g, respectively, at the end of 96 hours. The level in the cornea, sclera and aqueous humor at the end of 20 min after instillation of 0.2% ^{3}H-CSA was 1080, 176 and 13.7 ng eq/g or ml, respectively. The level in the cornea, sclera and aqueous humor at the end of 12 hours was 1340, 239 and 1.08 ng eq/g or ml, respectively. The level of CSA in the cornea, sclera and aqueous humor was reduced to 273, 6.72 and 0.63, respectively, at the end of 96 hours. The CSA level in lens and vitreous humor was 1.96 and 0.67 ng eq/g or ml , respectively, at the end of 20 min. The level was 1.45 and 0.53 ng eq/g or ml for lens and vitreous humor, respectively, at the end of 12 hours. The level of CSA in lens and vitreous humor was reduced to 2.45 and 0.13 ng eq/g or ml, respectively, at the end of 96 hours. The level of CSA in the iris-ciliary body was about 63.5 ng eq/g at about 20 min after the administration of ^{3}H-CSA. The level of CSA in the iris-ciliary body at 12 and 96 hours was 41.5 and 8.25 ng eq/g, respectively. The level of CSA in the lacrimal gland was 4.32, 8.90, 3.48 and 0.45 ng eq/g at 20 min, 1 hour, 12 hours and 96 hours, respectively. The level in the blood was about 0.65 ng eq/g at 0.3 hours to 0.52 ng eq/g at 48 hours. The level of CSA in the plasma at 0.3 hour and 48 hours was 0.58 and 0.47 ng eq/ml, respectively.

The maximum level in the tear from the untreated eye was [redacted] ng eq/g or ml at 0.67 hour. The radioactivity was present in the untreated eye possibly through the systemic circulation. Above data were collected from the female rabbits.

The distribution of CSA (measured as radioactivity) in the blood, plasma and ocular tissues in the treated and untreated eyes of male and female rabbits at selected time points is shown in the following tables.

Table VL Ocular tissue and blood concentrations in male versus female rabbits after a single ocular dose of a 0.2% ^{3}H-cyclosporine was applied to the left eye of rabbits.

Treated Eye Tissue/Fluid	ng-equiv/g or ml of tissue concentration (Mean ± SD, n=2-4)					
	0.67 hr		2.0 hr		24 hr	
	Female	Male	Female	Male	Female	Male
Tear	7940 ± 5680	3550 ± 1020	2830 ± 2430	1810 ± 240	515 ± 404	107 ± 12
Nictitating Membrane	1260 ± 550	1260 ± 378	811 ± 216	764 ± 194	82.8 ± 66.9	66.2 ± 53.0
Upper Conjunctiva	797 ± 193	1110 ± 650	705 ± 311	682 ± 130	36.9 ± 18.1	37.9 ± 12.2
Lower Conjunctiva	759 ± 300	827 ± 400	401 ± 124	577 ± 240	30.9 ± 20.8	34.6 ± 16.2
Cornea	1050 ± 430	964 ± 395	1070 ± 490	1200 ± 410	622 ± 308	667 ± 237
Sclera	154 ± 30	151 ± 47	185 ± 141	275 ± 147	34.5 ± 15.8	79.6 ± 48.1
Aqueous Humor	6.75 ± 2.38	6.12 ± 2.85	3.33 ± 0.92	3.76 ± 0.74	0.780 ± 0.241	0.931 ± 0.152
Iris-Ciliary Body	23.6 ± 7.9	52.3 ± 36.8	17.7 ± 5.4	23.8 ± 7.4	15.6 ± 7.4	28.0 ± 15.6
Lens	1.42 ± 0.93	2.22 ± 1.73	1.60 ± 1.04	1.33 ± 0.81	0.607 ± 0.193	1.57 ± 0.70
Vitreous Humor	0.428 ± 0.081	0.672 ± 0.075	0.271 ± 0.003	0.551 ± 0.241	0.215 ± 0.050	0.256 ± 0.069
Choroid/Retina	59.7 ± 27.4	83.6 ± 50.0	24.5 ± 11.5	59.4 ± 13.0	9.12 ± 6.44	23.5 ± 22.4
Optic Nerve	4.81 ± 4.30	1.43 ± 0.80	3.59 ± 3.47	1.99 ± 1.67	0.491[a]	ND[b]
Lacrimal Gland	7.78 ± 4.24	7.55 ± 1.34	7.34 ± 6.21	16.5 ± 7.9	1.87 ± 1.55	1.57 ± 0.35
Blood	0.639 ± 0.038	0.655 ± 0.196	0.674 ± 0.064	0.614 ± 0.118	0.560 ± 0.121	BLQ[c]
Plasma	0.443 ± 0.016	0.489 ± 0.106	0.631 ± 0.086	0.650 ± 0.168	0.445 ± 0.120	0.447 ± 0.085

[a] mean calculated for n= 2, undetectable radioactivity for 2 samples
[b] no radioactivity detected in 3 or more rabbit eyes in a group of 4
[c] below limit of quantitation

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Table VII. Ocular tissue and blood radioactivity concentrations in male versus female rabbits after a single ocular dose of a 0.2% ^{3}H-cyclosporine was instilled into the left eye of rabbit eyes.

Untreated Eye	ng-equiv/g or ml of tissue concentrations (Mean ± SD, n=2-4)					
Tissue/Fluid	0.67 hr		2.0 hr		24 hr	
	Female	Male	Female	Male	Female	Male
Tear	47.3 ± 21.5	24.1 ± 24.5	18.5 ± 12.5	21.3 ± 15.7	14.8 ± 7.7	7.71 ± 1.80
Nictitating Membrane	2.36 ± 0.74	0.421 ± 0.114	1.92 ± 0.56	1.25 ± 0.65	0.127[a]	0.284 ± 0.168
Upper Conjunctiva	2.22 ± 0.86	2.04 ± 1.70	1.61 ± 1.12	2.30 ± 0.90	ND[b]	ND
Lower Conjunctiv	4.57 ± 3.67	0.572 ± 0.439	1.54 ± 1.03	1.24 ± 0.86	ND	ND
Cornea	4.03 ± 0.67	0.317 ± 0.184	2.38 ± 1.38	1.38 ± 0.63	0.544[c]	ND
Sclera	2.12 ± 2.02	0.413 ± 0.477	1.44 ± 1.03	0.207 ± 0.094	ND	ND
Aqueous Humor[a]	0.411 ± 0.0278	0.415 ± 0.049	0.496 ± 0.101	0.387 ± 0.027	0.591 ± 0.143	0.452 ± 0.074
Iris-Ciliary Body	3.13 ± 0.35	1.77[c]	0.976 ± 0.523	0.299[a]	ND	ND
Lens	0.336 ± 0.273	0.257 ± 0.301	0.304 ± 0.112	0.0625 ±0.0422	ND	ND
Vitreous Humor	0.0441± 0.0104	0.0521± 0.0141	0.0778±0.0190	0.0616 ±0.0136	0.0775 ±0.0169	0.0498±0.0093
Choroid/Retina	1.20 ± 0.49	3.44 ± 2.06	4.18 ± 2.03	2.12 ± 1.02	1.92 ± 1.12	0.262 ± 0.206
Optic Nerve	4.27 ± 4.77	4.25 ± 4.10	0.851[a]	ND	ND	ND
Lacrimal Gland	1.30 ± 0.80	0.733 ± 0.399	0.886 ± 0.114	0.803 ± 0.476	0.420 ± 0.250	0.413 ± 0.118
Blood[a]	0.639 ± 0.038	0.655 ± 0.196	0.674 ± 0.064	0.614 ± 0.118	0.560 ± 0.121	BLQ[d]
Plasma[a]	0.444 ± 0.016	0.489 ± 0.106	0.631 ± 0.086	0.650 ± 0.168	0.445 ± 0.120	0.447 ± 0.085

[a] mean calculated for n = 2 in a group of 4 with outlier and undetected radioactivity
[b] no radioactivity detected in 3 or more rabbit eyes in a group of 4
[c] mean calculated for detected radioactivity in 2 rabbit eyes in a group of 4
[d] below limit of quantitation

R-1995-3430, p 120

The data suggest that CSA or its metabolites are bioavailable in the ocular tissues e.g. conjunctiva, nictitating membrane, lacrimal gland, cornea, iris, lens, vitreous humor, retina and optic nerve after the single dose. The systemic bioavailability of CSA was minimal after the single drop of ocular dose at 0.2%. There was no gender difference in the distribution in the ocular tissues. However, traces of radioactivity were observed in the untreated eye.

Ocular Cyclosporine distribution during 9 ½ days of dosing of 0.05% and 0.1% ^{3}H-Cyclosporine A emulsions to albino rabbit eyes. Report PK-98-074, Page 328, vol 19.

The study was conducted at Allergan, CA according to the GLP. The study period was between Oct 11, 1997 to Dec 2, 1999. 0.5% (Batch # 9054) and 0.1% (Batch # 8735X) ^{3}H-Cyclosporine emulsion batches were prepared for the study.

The experiment was conducted in female albino rabbits. There were two groups of rabbits. Each group comprised of 20 rabbits. Two rabbits were allotted to an untreated control. Animals were treated with one drop in both eyes twice a day with 0.05% or 0.1% ^{3}H-CSA emulsions for 9 days. Animals were treated once on the tenth day. Each drop was estimated to be 50 µL in volume.

Immediately before the last dose and at 0.33, 1, 3, 6 12, 24, 48, 96 and 144 hours after the dose, blood samples were collected from the ear vein. Tear samples were collected immediately after the sacrifice. Following tissues and fluids were collected from the eye:

Lacrimal gland, upper and lower conjunctivae, sclera, cornea, iris-ciliary body, lens, vitreous humour, choroid-retina and optic nerve head.

Radioactivity of the samples was detected by liquid scintillation counter.

Results:

Ocular PK parameters of ^{3}H-CSA after 9 1/2 days twice daily dosing in Albino rabbits.

C_{max} (ng eq/g), AUC $_{0-12}$ (ng eq.hr/g), T1/2 (hr) in the ocular tissues are presented in the following table.

Tissue	C_{max} 0.05%	AUC 0.05%	$T_{1/2}$ 0.05%	C_{max} 0.1%	AUC 0.1%	$T_{1/2}$ 0.1%
Tears	14000	82000	nd	41000	300000	27
Lacrimal gland	11.9	66	nd	15.4	140	nd
Upper conjunctiva	573	4420	nd	2020	16900	25.1
Lower conjunctiva	713	5030	nd	1920	15600	31.8
Cornea	1550	12300	50.9	4810	49300	52.0
Sclera	84.5	848	39.2	262	2710	40.5
Lens	18.4	186	480	55.2	529	271
Aqueous Humour	1.44	13.2	nd	7.19	60.4	nd
Vitreous humour	2.93	22.8	nd	10.2	87	nd
Optic nerve head	29.3	blq	nd	67.7	blq	nd
Iris ciliary body	74.7	672	57	246	2260	53.8
Blood	<0.694	BLQ		<1.88	blq	nd

blq = below limit of detection, nd= not determined

The whole blood to a plasma concentration ratio of ^{3}H Cyclosporine in mouse, rat, rabbit, dog and human in vitro.

Page 187, vol 18, # PK-94-108:

The CSA level in the blood is about 2-4 fold higher than the plasma due to uptake of the drug in red cells. However, the Pharmacodynamic and toxicity of CSA depend on the free plasma levels. The therapeutic levels after 3-10 mg/kg/day dose in humans are 50-150 ng/ml in plasma and 100-400 ng/ml in the whole blood. Approximately 41-58% of the drug is bound to the red cells, 4-9% in the leukocytes, 5-12% in the granulocytes and 33-47% found in the plasma. About 90-95% of the drug in plasma is protein bound. In the present study, the blood to plasma ratios of CSA was determined from whole blood samples that contained 0.5-10 ng/ml of CSA using mouse, rat, rabbit, dog and human blood **In vitro**. The results would provide information on the most susceptible species to the systemic exposures from the ophthalmic preparations.

^{3}H-CSA concentrations at 0.0580 μg/ml and 0.116 μg/ml were prepared and incubated with 2 ml of the whole blood and mixed for 30 sec. The final concentrations in the blood were 0.5, 1.0, 5.0, and 10.0 ng/ml. Six replicates were prepared for each concentration. Samples were shaken for 30 min at 37°C in the water bath. Liquid scintillation spectrometry and HPLC methods were used for the determination of Blood and plasma levels of radioactivity and CSA. Blood samples from mice and rats were obtained by venipuncture. Blood samples from rabbits were collected from the ear vein and fresh human venous blood was collected from human volunteers.

Results:

In a preliminary experiment, it was shown that blood to plasma ratios was about 1.7 over six-hour period **in vitro** suggesting that the equilibrium exists for a long time between the blood cells and plasma proteins. Temperature effect was noticed for the blood to plasma ratios between the species as shown in the following table.

Temperature	Blood Levels	Mouse	Rat	Rabbit	Dog	Human
	ng/ml	Ratio	Ratio	Ratio	Ratio	Ratio
20°C	0.5	2.58	0.96	1.43	1.26	1.61
20°C	1.0	2.83	1.07	1.62	1.22	1.83
20°C	5.0	3.01	1.12	1.67	1.17	1.97
20°C	10.0	3.05	1.17	1.77	1.29	2.00
37°C	0.5	1.84	0.9	1.31	1.00	1.49
37°C	1.0	1.96	1.04	1.41	1.08	1.61
37°C	5.0	2.09	1.06	1.48	1.09	1.72
37°C	10.0	2.15	1.10	1.55	1.11	1.75

Results of the experiment suggest that rabbit and human had comparable blood to plasma ratios. A similarity was also observed for rat and dog blood. However, blood to plasma ratios in the mouse was the highest. The blood to plasma ratio of radioactivity was reduced at 37°C compared to that at 20°C. Therefore, plasma level of the drug is greater at 37°C compared to 20°C.

Dose proportionality for ocular distribution of 0.05, 0.2 and 0.5% ophthalmic emulsion of ^{3}H-Cyclosporine in NZ rabbits. Page 075, vol 19, Report# PK-96-011:

The objective of the study was to find dose proportionality to ocular bioavailability of a single drop of 0.05%, 0.2% and 0.4% ^{3}H-Cyclosporine in each eye. The lot # for CSA was 91272 and that for ^{3}H-CSA was 7225. The purity of non-labeled and ^{3}H-CSA was 99.7% and 98%, respectively. Female NZ white rabbits 3-4 months of age and 1.7-2.4 kg body weight

were used in the study. Groups of three rabbits per dose per time point were used for the study. Rabbits were sacrificed at 0.33, 1, 3, 6, 8 and 24 hours after the dose. Prior to the sacrifice one ml of blood was collected from each rabbit. Following sacrifice, tissues and samples were collected for the determination of radioactivity in upper conjunctiva, lower conjunctiva, cornea, aqueous humor, iris-ciliary body and lacrimal gland. Two rabbits were allotted for control tissues.

Results:

Blood levels:

Except one-hour sample at 0.2% dose that had 0.785ng eq/g, all other samples showed CSA levels below the level of quantitation of

The C_{max} (ng eq/g), T_{max} (hr) and AUC $_{0-24}$ (ngeq.hr/g) in the ocular tissues are shown in the following table.

Tissues	C_{max}			T_{max}			AUC $_{0-24}$ hr		
	.05%	.2%	.4%	.05%	.2%	.4%	.05%	0.2%	0.4%
Up. Conjunc.	1320	2610	2750	0.3	0.3	0.3	5560	12400	15000
Low. Conjunc.	946	2180	2840	0.3	0.3	0.3	4690	9730	14900
Cornea	873	2650	4050	8	8	8	14100	43300	73300
Aq. Humor	0.8	1.99	4.8	8	0.3	0.3	12.5	30.4	69.4
Iris-Ciliary	13.7	32.9	85.9	8	8	0.3	246	675	1220
Lacr. Gl.	6.16	32.7	53.3	0.3	0.3	0.3	64.4	332	493

The radioactivity in the cornea increased with the dose and with the increase in the sampling time. Data suggest that the maximum radioactivity was observed in the cornea after 6-7 hours following dosing.

Three different concentrations of the ophthalmic formulation showed an increase in the levels of Cyclosporine in the cornea, lacrimal gland, upper conjunctiva, lower conjunctiva, aqueous humor and iris-ciliary body. The increase was not dose proportionate. A plot of dose normalized AUC in the upper conjunctiva, lower conjunctiva, cornea and iris-ciliary body showed negative slope. The dose normalized AUC plot for aqueous humor and lacrimal gland at several concentrations of ^{3}H-CSA was flat. Data suggest all dosage forms were bioavailable to ocular tissues. There was no dose proportionality to the distribution of ^{3}H-CSA in the ocular tissues.

Absorption and distribution of 0.2% ^{3}H-cyclosporine emulsions multiple doses in male beagle dogs. Page 100, vol 19, PK-96-016:

The study was conducted at The study was conducted according to the GLP. The study period was from Dec 1-7, 1995 and animals were sacrificed within Dec 11, 1995.

Lot # of ^{3}H-Cyclosporine was 7478 and radiochemical purity was 98.8%. Non-labelled Cyclosporine lot# was 91272-282N. Six to seven months old male beagle dogs weighed 9.7 -12.9 kg were used in the study.

^{3}H-Cyclosporine (CSA) emulsion was administered one-drop (35 µL) in each eye twice daily for 7 days in seven dogs. One of the seven dog was sequentially sacrificed at 1, 3, 6, 12, 24, 48 and 96 hours after the final dose. Another dog was treated one drop twice daily in the right eye only. The dog was sacrificed one hour after the final dose. Blood and tear samples were collected immediately before sacrifice. Following tissues and fluids were collected after the sacrifice.

Conjunctiva, lacrimal gland, nictitating membrane (gland), optic nerve, orbital fat, and muscle, aqueous humour, vitreous humour, cornea, iris-ciliary body, lens, sclera, chroid and retina.

The total radioactivity in the ocular tissues, blood and plasma were determined by scintillation counter. Clinical signs were also noted on a regular basis for any abnormality. Any spillage of the dosed emulsion after the applications was collected in the cotton tipped applicator for the assay of radioactivity.

Results:

Animal #6 showed discharges from both eyes and slight inflammation from the left eye from day 4 of dosing. No other animals showed clinical signs of toxicity.

Radioactivity as the ng eq/g of tissues for several tissues is presented in the following table.

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TABLE 1

Concentrations of radioactivity (ng equivalents cyclosporine/g) in ocular tissues, tears, plasma and blood after administration of multiple ocular doses of ^{3}H-cyclosporine (0.2% w/w) emulsion to beagle dogs for 7 days

Tissue	1d		2d		3d		4d		5d		6d		7d	
	1 hour		3 hours		6 hours		12 hours		24 hours		48 hours		96 hours	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Tear	16426	32998	36487	20973	14561	8352	4493	1913	5326	1768	1531	255	247	1157
Conjunctiva	2739	1275	1712	947	1071	607	175	308	313	187	2.51	147	22.5	78.2
Cornea	1818	1800	1358	655	1303	1677	820	1213	953	855	321	952	418	650
Sclera	119	132	58.5	73.8	23.2	41.6	28.7	26.0	20.0	18.4	2.48	34.0	21.3	29.5
Aqueous humour	0.61	0.62	0.90	0.36	0.40	0.36	0.33	0.79	0.14	0.25	0.19	0.38	0.28	0.51
Iris/ciliary body	12.7	18.2	44.7	26.6	33.6	33.8	15.4	32.8	21.6	21.1	11.2	32.2	18.3	19.7
Lens	1.81	4.13	2.43	2.36	3.32	2.76	1.76	1.30	1.49	1.44	1.71	2.51	2.56	3.44
Vitreous humour	0.32	0.19	0.46	0.11	0.15	0.26	ND	0.17	0.10	0.15	ND	0.35	0.14	0.32
Choroid/retina	7.54	13.6	21.1	3.58	9.68	5.61	4.86	5.56	3.69	2.31	2.47	5.25	7.73	8.46
Optic nerve	7.92	10.4	7.69	5.18	3.31	4.84	1.91	2.59	1.79	5.55	1.34	4.52	8.40	16.6
Nictitans gland	1049	331	411	212	516	522	132	167	114	65.5	2.82	25.7	8.57	26.7
Lacrimal gland	160	321	518	196	151	111	45.5	99.8	62.3	78.9	4.17	27.7	10.1	10.9
Orbital fat	25.0	39.8	17.7	80.6	10.6	15.0	21.5	33.0	8.05	21.6	3.36	6.54	5.29	58.4
Eye muscle	28.3	30.4	36.6	6.94	7.89	15.9	2.22	4.26	6.86	9.86	1.21	3.19	4.65	147
Blood	1.15		1.00		0.41		0.51		0.24		0.36		0.36	
Plasma	0.33		0.25		0.13		0.25		0.12		ND		0.09	

ND Below the limit of accurate determination (<2 x background)
Limits of detection are as follows: (ng equivalents/g)

Aqueous humour	(8d, left)	0.58				
Vitreous humour	(4d, left)	0.21	(6d, left)	0.20	(8d, left)	0.58
Plasma	(6d, left)	0.14	(8d, left)	0.29		

ALG 33/960262

EEC SUMMARY TABLE

Name of company: Allergan Inc	TABULATED STUDY REPORT	
Name of Finished Product:	Ref to III.G.310	
Name of active ingredient: Cyclosporine (cyclosporin A)	Page 1 of 1	

PHARMACOKINETICS: Pharmacokinetics after repeated administration

Ref to Document:	Volume: 1	Page to	Addendum No. -
Report date:	August 1996	Number: ALG 33	Study period (years): 1995 - 1996

Species/Strain:	Beagle dogs
No. of animals:	7 males dosed in both eyes and 1 male dosed in one eye
Dosage:	37 μCi/79.1 μg/35 μl
Administration:	Single eye drop (35 μl)
Administrations/day:	Twice daily
Duration of treatment:	7 days
Formulation:	0.2% w/w emulsion (Formulation No. 8734X-H3)
Analytical methods:	Liquid scintillation analysis
Nuclide:	^{3}H
Specific radioactivity:	1.06 mCi/2.26 mg cyclosporine

Plasma/blood	Blood	Plasma
C_{max} (ng equiv/g):	1.15	0.33
T_{max} (h):	1	1
AUC (0-t) (ng equiv.h/g):	36.0	12.1
CL/f:	NC	NC
$t_{½}$:	NC	NC
Binding % bound:	NA	NA

NA = Not applicable NC = Not calculable

Summary of tissue kinetic data (mean values)

Sample	T_{max} (hours)	C_{max} (ng equiv/g)	$t_{½}$ (hours)
	Mean	Mean	Mean
Tear	1	288730	NC
Conjunctiva	1	2007	34.1
Cornea	1	1809	NC
Sclera	3	125	NC
Aqueous humour	3	0.63	NC
Lens	3	35.7	NC
Vitreous humour	6	3.04	NC
Iris/ciliary body	3	0.28	NC
Choroid/retina	1	12.3	NC
Optic nerve	3	12.5	NC
Nictitans gland	3	690	17.7
Lacrimal gland	96	357	28.0
Orbital fat	96	49.2	NC
Eye muscle	3	75.8	NC

NC = Not calculable
These tissues had an apparently long terminal elimination phase such that their terminal half-lives could not be calculated.

Excretion, recovery: Radioactivity (% of dose) NA

Study conducted by the applicant: Yes No/
Study conducted in the laboratories of

Study in compliance with GLP: Yes/ No Not required

Levels of radioactivity in tear, conjunctiva, cornea, sclera, lacrimal and nictitating glands were higher compared to lens, retina and vitreous humor. Whole blood levels of radioactivity were around 1 ng/ml equivalent or less. Those animals treated in one eye only showed radioactivity in the contralateral eye presumably via systemic circulation. Data for the ocular tissues and blood for the dog (#8) that was treated in the right eye only and the data for the dog (#1) that was treated in the both eyes are shown in the following table.

Concentrations of radioactivity (ng equivalents cyclosporine/g) in ocular tissues, tears, plasma and blood after administration of multiple ocular doses of ^{3}H-cyclosporine (0.2% w/w) emulsion to beagle dog in the right eye only, after one hour

Tissue	8♂			1♂
	1 hour			1 hour
	Left (untreated)	Right (Treated)	Right (treated)/ Left (untreated)	Right/ Left
Tear				
Conjunctiva				
Cornea				
Sclera				
Aqueous humour				
Iris/ciliary body				
Lens				
Vitreous humour				
Choroid/retina				
Optic nerve				
Nictitans gland				
Lacrimal gland				
Orbital fat				
Eye muscle				
Blood				
Plasma				

ND Below the limit of accurate determination (<2 x background)
Limits of detection are as follows: (ng equivalents/g)

Aqueous humour	(8♂, left) 0.58
Vitreous humour	(8♂, left) 0.58
Plasma	(8♂, left) 0.29

Appendix 1

NDA 50-735

1 INTRODUCTION

Sandimmune® (cyclosporine) is an FDA-approved drug for use in the prophylaxis of organ rejection in kidney, liver, and heart allogenic transplant patients. Provided below is a brief summary of the findings in the studies performed to date on cyclosporine, including new findings from the literature and selected reference articles. Various studies performed on cyclosporine and submitted in the original NDAs (i.e., NDA 50,573 and 50,574) are described in Ryffel, et al. (1983).

2 TOXICOLOGY

2.1 Acute Toxicology Studies

Acute (i.e., single-dose administration) oral toxicity evaluations (Document No. 203-003; Amendment No. 1, Document No. 203-036) were conducted with cyclosporine in mice, rats, and rabbits. Acute intravenous toxicity studies in mice (Document No. 203-021), rats (Document No. 203-022), and rabbits (Document No. 203-024) were also conducted with a Cremophor EL intravenous formulation of cyclosporine. Clinical signs of overdosage that were observed immediately following cyclosporine administration included hyperventilation, drowsiness, diarrhea, muscular spasms, and piloerection. Body weight loss was also apparent after oral administration. The mean LD_{50} values (95% confidence limits) following oral administration were: mice, 2329 mg/kg (1848-3020); rats, 1480 mg/kg (1105-1997); and rabbits, >1000 mg/kg. The mean LD_{50} values (95% confidence limits) following intravenous administration were: mice, 107 mg/kg (could not be calculated); rats, 25 mg/kg (22-29); and rabbits, >10 mg/kg.

2.2 Multidose Toxicology Studies

2.2.1 78-Week Oral (Diet) Study in Mice

A 78-week oral (diet) carcinogenicity study was conducted in mice at 0, 1, 4, or 16 mg/kg/day (Document No. 203-025). At 4 mg/kg/day, anemia and reticulocytosis were observed in a limited number of animals. At 16 mg/kg/day there was increased mortality; females were more affected. Hematological examinations revealed anemia (4/20 animals) and lymphocytic leucocytosis with atypical lymphocyte forms (1/20 animals). In addition, there was a trend toward fewer thrombocytes (3/20 animals). Histopathological examination did not reveal any dose-dependent, treatment-related adverse effect on the incidence of neoplastic or non-neoplastic lesions. However, a histopathological re-evaluation (Document No. 203-251) revealed that the incidence of hepatocellular carcinomas in males at 4 mg/kg/day (10/50) exceeded the incidence in controls (7/50); this finding was not dose-dependent, i.e., the incidence at 16 mg/kg/day was 6/50. Additionally, re-evaluation concluded that there was a treatment-related increase in the incidence of malignant lymphoma at 16 mg/kg/day (males: 23/50; females 29/50) compared to that of controls (males: 16/50; females: 21/50) was apparent. Malignant lymphomas in animals receiving 1 or 4 mg/kg/day was comparable to that of control.

2.2.2 13-Week Oral (Diet) Study in Rats

In a 13-week subchronic toxicity study, rats (10/sex/group) were administered cyclosporine orally (diet) at 14, 45, or 90 mg/kg/day (Document No. 203-005). Mortality was 30% at 45 mg/kg/day and 90% at 90 mg/kg/day. Signs of hepatic and renal toxicity were observed. After a recovery period (6 weeks), BUN and serum glutamate oxaloacetate transaminase values were similar to normal values. Postmortem examination after 13 weeks of cyclosporine administration revealed microscopic evidence of renal and hepatic toxicity at 45 and 90 mg/kg/day, and included vacuolation, necrosis, and regeneration of proximal tubular epithelium in the kidney, and fatty change, degeneration and single- cell necrosis of hepatocytes in the liver. There was a slight increase in the incidence of small inflammatory lesions and atrophy of lymphatic tissues (lymph nodes, spleen, and thymus). Hematologic changes consisted of slight anemia, leukocytosis with neutrophilia and lymphopenia, monocytosis, and eosinopenia, which were reversible during the 6-week recovery period. Based on the results of this study, the no-toxic-effect level (NTEL) was 14 mg/kg/day.

2.2.3 2-Year Oral (Diet) Study in Rats

A 2-year oral (diet) chronic toxicity/carcinogenicity study in rats was performed at doses of 0, 0.52, 2.1, or 8 mg/kg/day (Document No. 203-030). At 8 mg/kg/day, a distinct inhibition of weight gain, decreased food consumption, and high mortality rates (treated - males: 47/50, females: 39/50; control - males: 35/50, females: 33/50) were observed. Females at 2.1 mg/kg/day exhibited decreases in body weight gain (25%) and food intake (3%) and slight increases in mortality (males: 36/50; females: 33/50), compared to that of controls (males: 36/50; females: 38/50). At 2.1 and 8 mg/kg/day, slight anemia (males) and slight neutropenia and lymphopenia (males and females) were observed; the leukocyte values were similar to control levels by the end of the study. Treated males showed slight hepato- and nephrotoxic effects as evidenced by increases in alkaline phosphatase, glutamic pyruvic transaminase, glutamate oxaloacetate transaminase, creatinine, and BUN, and a decrease in total protein. Histological evaluation of tumors did not reveal dose-dependent increases in tumors nor a different pattern compared with controls. The incidence of pancreatic islet cell adenomas in males at 0.52 mg/kg/day (5/50) significantly exceeded control values (2/50), but this effect was not observed at higher doses. Based on the results of the chronic toxicity phase of this study, 0.52 mg/kg/day was an NTEL. A histopathological re-evaluation (Document No. 203-252) of the tissues from this study resulted in the same conclusion as the original study report, i.e., cyclosporine does not possess carcinogenic potential.

2.2.4 52-Week Oral (Capsule) Study in Dogs

A 52-week oral chronic toxicity study was performed in Beagle dogs at doses of 0, 5, 15, or 45 mg/kg/day cyclosporine administered in olive oil (Document No. 203-018). Swollen gums were observed at 15 and 45 mg/kg/day. Histopathologic evaluation of the swollen gums revealed chronic nonspecific periodontitis and gingivitis with abundant plasma cell infiltration, which were also observed in control animals. At 5 mg/kg/day, a slight increase in erythrocyte sedimentation rate and slight decreases in serum protein and albumin were detected. Hematological evaluations revealed a slightly lower mean eosinophil value and

a slight leukopenia in one dog. Clinical chemistry evaluations revealed slightly higher serum lipid values, lower serum albumin, reduced serum protein values, and slightly elevated serum globulin values in 3/8 of animals at 15 mg/kg/day.

At 45 mg/kg/day, in addition to the above findings, a decrease in leukocyte counts and distinct anemia in two dogs were attributed to malnutrition. Some atypical lymphocytes were also found. Dogs receiving 45 mg/kg/day (5/8) had a widely distributed papillomatosis, primarily between Weeks 20 and 40. By the end of treatment, all papillomata had regressed except for that seen in one male dog. The histology of the lesion was similar to canine oral papillomatosis (Chambers, et al., 1970), without the correlation of viral particles or confirmatory immunological analysis. Additionally, one skin fibroma at 15 mg/kg/day and one skin basalioma at 45 mg/kg/day were observed in two dogs (total). Tumors of this type spontaneously occur in dogs (Miller, et al., 1976). Cystic nodules of undetermined etiology were found on the pericardium and diaphragm of two dogs; these were not neoplastic but resembled rheum nodules morphologically. In some treated dogs there was atrophy of lymphoid organs and evidence of slight regeneration of tubular epithelium in the kidneys. At 15 mg/kg/day, one dog had a mononuclear cell infiltration and enlargement of the regions surrounding the portal vein of the liver. A spontaneous regression of the papillomatosis that was observed toward the end of the drug administration period continued during the 12-week recovery phase. Swelling of the gums and biochemical and hematological alterations were also fully reversible. Based on the results of this study, 15 mg/kg/day was an NTEL.

2.2.5 13-Week Oral (Capsule) Study in Monkey

A 13-week oral (capsule) subchronic toxicity study in rhesus monkeys was performed at 0, 20, 60, or 200/300 mg/kg/day (Document No. 203-006). At 60 mg/kg/day, a transient decrease in total leukocyte count was observed at Week 4, which returned to normal by Week 13. A slightly impaired weight gain was noted at 200 mg/kg/day (which was escalated to 300 mg/kg/day for the last 4 weeks due to a lack of toxicity). Hematologic examinations, including bone marrow evaluations, were normal. Variable atrophy of the lymphatic tissues at the higher doses was observed. Evidence of liver (hepatocyte swelling and fatty infiltration) and kidney toxicity were observed at 200/300 mg/kg/day. Dose-dependent increased incidences of dilated tubuli atrophy of the lymphoid follicles of the spleen in cyclosporine-treated monkeys indicated a pharmacodynamic effect. Based on the results of this study, the NTEL was set at 60 mg/kg/day.

2.2.6 Additional Information

Experimental and clinical data from the literature which are relevant to the evaluation of the carcinogenic potential of the immunosuppressant, cyclosporine, have been reported previously (Ryffel, 1992; Ryffel, et al., 1992). Excessive immunosuppression may allow expression and growth of initiated or transformed cells which are already present (Hanto, et al., 1985). Review of the data regarding the carcinogenicity of cyclosporine in humans indicates that higher doses of this drug, particularly in combination with other immunosuppressants, can produce sufficient pharmacologic immunosuppression to result in malignant diseases. Various immunosuppressive agents have been associated with an increased incidence of lymphoproliferative disorders and other malignancies, particularly of the skin. These lymphoproliferative lesions may regress after dose-reduction or cessation of treatment (Starzl, et al., 1984). This risk may be reduced or

eliminated by maintaining the lowest effective dose. Clinical studies with a reduced dosage of cyclosporine as a single immunosuppressive agent have provided efficacious immunosuppression without a significant increase in tumor incidence. At the lower dose of cyclosporine used for autoimmune disease or kidney transplantation, the incidence of malignant disease is similar to conventional immunosuppressant treatments (Cockburn, 1987).

Administration of immunosuppressive drugs also has the potential to augment UV light-induced skin carcinogenesis (Daynes, et al., 1979). Exposure to ultraviolet light produces skin cancer in hairless mice (Gallagher, et al., 1984). Co-administration of cyclosporine (subcutaneously at doses of 10 or 25 mg/kg thrice weekly, for up to 24 weeks) or azathioprine (intraperitoneally at doses of 4 or 8 mg/kg thrice weekly) resulted in a shortening of the latency period, a higher percentage of surviving animals developing tumors, and multiple tumors on a single animal in this model (Nelson, et al., 1987). Immunosuppressive drugs administered to hairless mice, at doses similar to maintenance levels for transplant recipients (based on body surface area), may enhance UV light-induced skin carcinogenesis (Kelly, et al., 1987). Cyclosporine administration (orally at a dose of 60 mg/kg/day) caused a moderate reduction in the latent period for tumor induction, while azathioprine (intraperitoneally at a dose of 15 mg/kg/day) and cyclophosphamide (intraperitoneally at a dose of 15 mg/kg/day) reduced tumor latency periods and increased the numbers of tumors/animal. However, prednisolone (intraperitoneally at a dose of 20 mg/kg/day) administration did not affect the onset or incidence of UV light-induced skin carcinogenesis. Thus, these studies support the hypothesis that immunosuppressive drugs may augment UV light-induced skin carcinogenesis in hairless mice.

2.4 Reproduction Studies

2.4.1 Fertility and Reproductive Performance

A fertility and general reproductive performance study was performed in rats (Document No. 203-027). Animals of the F_0 generation were treated orally with doses of 0, 1.5, 5, or 15 mg/kg/day; they were treated for 9 (males) or 2 (females) weeks prior to mating and continuing for 62 days (males) or until necropsy (females). Prolonged treatment with 5 or 15 mg/kg/day produced in-life and postmortem effects in F_0 males, as well as reduced body weight gain; no effects were observed at 1.5 mg/kg/day. Females were affected only at 15 mg/kg/day; two dams showed dystocia and had to be sacrificed moribund. The reproductive performance of F_0 animals was normal, except for an increased perinatal mortality and a possibly impaired postnatal development of F_1 pups in single litters at 15 mg/kg/day. No adverse effects were noted at the lower doses. There were no effects on fertility of the F_1 animals and the development of their offspring was normal.

2.4.2 Embryofetal Development

A teratology study was performed in pregnant rats [redacted] at doses of 0, 10, 17, 30, 100, or 300 mg/kg/day administered in 2% gelatin from Days 6-15 after copulation (Document No. 203-008). Doses up to 17 mg/kg/day were well-tolerated by the dams. Weight gain was impaired at 30 mg/kg/day and weight loss was observed at 100 and 300 mg/kg/day. Maternal mortality was observed at 300 mg/kg/day. Prenatal mortality accompanied maternal toxicity at 30 mg/kg/day and higher. There was no evidence of a

teratogenic effect of the compound.

Another teratology study was performed in pregnant rabbits at doses of 0, 10, 30, 100, or 300 mg/kg/day administered orally in 2% gelatin from Days 6 to 18 postcoitus (Document No. 203-007). Dose levels of 10 and 30 mg/kg/day were well-tolerated, while administration of 100 and 300 mg/kg/day resulted in dose-dependent body weight loss. In addition, approximately one-half the females in these groups had enlarged mammary glands; histopathological evaluation revealed no effects on lactating glands. Reproductive parameters were not affected at 10 or 30 mg/kg/day. At 100 and 300 mg/kg/day, the compound proved to be embryo- and fetotoxic, but not teratogenic, compared to controls.

2.4.3 Peri- and Postnatal Development

A peri- and postnatal study was performed in female rats at orally administered doses of 5, 15, or 45 mg/kg/day from Day 15 postcoitus until necropsy (Day 21) (Document No. 203-023). At 45 mg/kg/day, body weight gain was reduced in the dams during the last third of gestation and increased peri- and postnatal mortality was observed in the offspring. No adverse effects were observed at 5 or 15 mg/kg/day.

2.4.4 Additional Information

Mice (A/J strain), administered 50 mg/kg/day of cyclosporine intraperitoneally, on Day 12 of gestation exhibited significantly more fetal resorptions, a lower incidence of cleft lip and palate, and a low frequency (<8%) of isolated cleft palate, compared to controls (Gasser, et al., 1992). These results suggest that embryotoxic doses can alter fetal development, possibly by inhibiting fetal growth.

Fetotoxicity, characterized by fetal mortality or runting, was observed in rats administered cyclosporine at 25 mg/kg/day immediately prior to mating and through Day 20 postcoitus (Mason, et al., 1985). This dose also produced maternal toxicity in the kidney, and immunosuppression and atrophy of the thymus, spleen, and lymph nodes.

In another study (Classen and Shevach, 1991), intravenous administration of cyclosporine to pregnant mice at 11 mg/kg/day during the peri- and postnatal phase resulted in detectable fetal tissue levels and equivocal effects on the fetal immune system. In offspring of dams treated at this dose, autoantibodies and an increase in splenic T cells were observed. These alterations were attributed to effects on the immune system of the developing fetus, suggesting possible immunosuppression.

Cyclosporine at 10 mg/kg/day, a dose which resulted in maternal renal and pancreatic β-cell toxicity, produced a reduction in the number of newborns/mother, and vacuolation of newborn kidney proximal tubule cells and pancreatic β-cells (Papaccio and Esposito, 1990). These results also suggest that maternally toxic doses can be embryotoxic.

Cyclosporine (30 mg/kg/day) administered to ICR mice produced maternal histopathological alterations in the thymus, liver, kidney, and spleen (Fein, et al., 1989). The findings of a reduced number of viable embryos and increased number of resorbed embryos indicated that cyclosporine has an embryotoxic potential; no teratogenic potential was observed.

Additional information regarding cyclosporine toxicity in the reproductive organs of male rats is also available in published nonclinical studies. Administration of 40 mg/kg/day of cyclosporine to rats resulted in impaired testicular function, i.e., decreased sperm counts and decreased fertility (Seethalakshmi, et al., 1990). These effects were reversed by co-administration of human chorionic gonadotropin. In another study, lower doses (20 mg/kg/day) administered to rats for 30 days produced a decrease in blood testosterone levels and atrophy of Leydig cells (Cavallini, et al., 1990). The dose levels used in these studies were in excess of those that produced toxicity (decreased body weight gain and nephrotoxicity) in a fertility and general reproductive performance study in Wistar rats (Ryffel, et al., 1983). At these toxic dose levels there was no effect on fertility.

2.5 Genotoxicity Studies

No genotoxic activity or DNA-binding have been detected with an array of tests (Zwanenburg, et al., 1988) which include: *in vitro* mutagenicity evaluations using *Salmonella typhimurium* (Document Nos. 203-004 and 203-039) and V79 Chinese hamster cells (Document No. 203-008); an *in vitro* micronucleus test in mice (Document No. 203-009); an *in vivo* cytogenetic analysis of Chinese hamster bone marrow cells for evaluation of clastogenic potential (Document No. 203-026); a dominant lethal test using male mice for evaluation of mutagenic potential (Document No. 203-029); an *in vivo* unscheduled DNA synthesis assay using male mice sperm heads (Document No. 201-006); and an *in vitro* evaluation of the induction of chromosomal aberrations using human peripheral blood lymphocytes (Document No. 203-292).

A study analyzing sister chromatid exchange (SCE) induction by cyclosporine in human lymphocytes *in vitro* (Yuzawa, et al., 1986), provided an indication of a slightly positive effect (i.e., induction of SCE) at high concentrations (e.g., 1 or 5 µg/mL). The mean SCE/cell for test article groups was 8.44, 9.02, 11.17, and 14.28 for 0.04, 0.2, 1, and 5 µg/mL groups, respectively, and 8.10 and 19.82 for the negative and positive controls, respectively. For a full interpretation of these results, it is necessary to evaluate the cytotoxicity data. At these levels, i.e., 1 and 5 µg/mL, the mitotic index was reduced 100-fold. This steep dose-response curve made the selection of the highest test compound concentration very difficult. The mitotic indices were highly variable, therefore, it cannot be excluded that 1 and 5 µg/mL doses exceeded an acceptable level of toxicity in terms of true cell killing. This may be indicative of an unusually strong cytotoxic effect.

3 SUMMARY AND CONCLUSIONS

The toxicology studies and literature references cited indicate that cyclosporine is a potent immunosuppressant that causes atrophy of lymphoid tissues at higher dose levels, but does not elicit myelotoxicity. Cyclosporine is not mutagenic at noncytotoxic concentrations and shows no evidence of DNA-binding; however, excessive immunosuppression can allow growth of transformed cells. Additional potential target organs for cyclosporine toxicity include the liver and kidney. Most of the pharmacodynamic and toxicologic effects of cyclosporine have been shown to be reversible upon withdrawal of compound. No potential for teratogenic effects in either species was observed in rats and rabbits at the dose levels administered (up to 300 mg/kg/day). Embryo/fetotoxic or peri-/postnatal toxicity were observed only at maternally toxic doses (rats: 30 mg/kg/day; rabbits: 100 mg/kg/day).