CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-744

PHARMACOLOGY REVIEW(S)

540 BLATT

NDA 50-744

Review and Evaluation of Pharmacology and Toxicology Data
Division of Dermatologic and Dental Drug Products (HFD-540)

JAN - 4 1997

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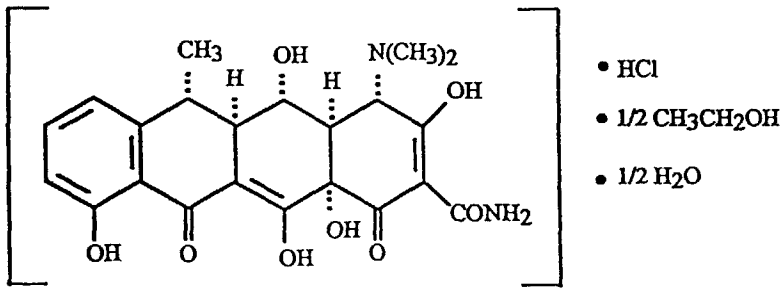
Initial Draft Completed: 12/6/96
Final Version Completed: 12/9/96

Original Summary
Submission Date: 8/30/96
Center Receipt Date: 8/30/96

Sponsor: Collagenex Pharmaceuticals

Drug: Periostat; doxycycline hyclate capsules, USP

Structure:



Formulation: Hard capsules that contain:

Doxycycline hyclate, USP.....23.08mg*
Microcrystalline Cellulose, NF.....177.00
Magnesium stearate, NF.....1.00

*Equivalent to 20.0mg doxycycline

Proposed Indication: Periodontitis

Related Drugs/INDs/NDAs: IND

Maximum Recommended Dosage: Two capsules per day (40mg doxycycline per day, or 0.8mg/kg/day in a 50kg individual; expressed as the salt, the dosage is 46.16mg doxycycline hyclate per day, or 0.92mg/kg/day)

Background Notes: This application pertains to the use of low dosages of doxycycline hyclate as a collagenase inhibitor to treat periodontitis. The rationale behind the product is that excessive collagenase activity, with consequent loss of

connective tissue, is a factor in the progression of periodontitis. Hypothetically, reduction in the activity of collagenase may slow progression and/or permit reversal of the disease. Collagenase is apparently dependent on calcium and zinc ions, and doxycycline may act through ion chelation. Note that an antimicrobial effect is not the proposed mechanism of action.

During a meeting with the sponsor on 12/21/95, division and office-level personnel agreed to waive the need for certain studies and to permit other studies to be "phase-4" commitments, partially on the basis of the clinical experience that is available to support the safety of doxycycline. Specifically:

Table of Contents: **Page**

1. Pharmacodynamics. Doxycycline hyclate is an antibiotic compound with activity against many species of bacteria when administered at sufficient dosage. Please see the approved label for NDA 50-006 (Vibramycin) for a list of the species against which doxycycline has been proven to be effective. The proposed label for Periostat recommends a dosage of 20mg doxycycline twice daily. This dosage is apparently below the threshold for antibacterial effects. The speculative mechanism of action of Periostat in the treatment of periodontitis is inhibition of human collagenase. Collagenase, a metalloproteinase, is postulated to be involved in the pathogenesis of periodontitis. Doxycycline has been demonstrated to inhibit the activity of collagenase obtained from damaged rabbit corneas; the *in vitro* IC_{50} was estimated to be 15μM.

2. ADME, Pharmacokinetics. The pharmacokinetics of doxycycline have not been studied in animals under the IND that led to this NDA (IND . However, the "ADME" properties of doxycycline have been investigated and published[1]. Doxycycline is generally well absorbed from the GI tract, although aluminum-containing antacids will interfere with absorption. Apparently, the extent of protein binding is variable, ranging from %. Published values for the serum half-life and renal clearance of doxycycline are 14.5-22 hours and 16ml/min., respectively. The primary route of elimination of doxycycline is excretion in the bile; elimination of doxycycline is apparently not compromised by renal dysfunction.

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[1]American Hospital Formulary Service, American Society of Hospital Pharmacists, Washington, D.C.

3. Toxicology.

3.1 Acute Toxicity.

3.1.1 Doxycycline hyclate: Acute oral toxicity study in the rat (MTD determination and limit test), study No. CNX/8/95, report dated 7/11/96, conducted by
in compliance with Good Laboratory Practice regulations (21 CFR 58).

This study was performed to evaluate the effects of acute exposure to doxycycline. The study was performed in two parts using Crl:CD(SD)BR rats. The first portion consisted of a maximum-tolerated-dose (MTD) determination and involved four male and four female rats divided into two groups. The test article, doxycycline hyclate (lot No. MA51TJ0503) was dissolved in water and administered by gavage at dosages of 500mg/kg and 750mg/kg. These animals were observed for 14 days after which they were discarded without necropsy. This phase was followed by a limit test where a group of five males and five females received the test article at a dosage of 500mg/kg. These animals were observed for 14 days and body weights were recorded on days 1, 8 and 15. At the end of the observation period surviving animals were killed and subjected to a gross necropsy. Animals in both phases were fasted overnight prior to dosing.

Results. During the initial (range finding) study, one male animal at a dose level of 750mg/kg was found dead on the third day following dosing. Clinical signs noted in this animal on day two included hypoactivity and piloerection. All other animals were unremarkable during this phase. It was concluded from the range finding phase that a dose level of 500mg/kg could be used for the limit test. No unscheduled deaths occurred during the limit test, and all animals were unremarkable throughout the study. No abnormal macroscopic findings were noted during necropsy.

Conclusions. It was concluded that doxycycline hyclate did not elicit signs of toxicity when administered to Crl:CD(SD)BR rats by gavage on a single occasion at a dosage of 500mg/kg. Of two males and two females that received single doses of 750mg/kg, only one death occurred (a male).

3.2 Multiple Dose Toxicity.
As noted on page two of this review, division and office-level personnel agreed in a meeting with the sponsor on 12/21/95 that may be regarded as phase-4 commitments. The sponsor has committed to performance of and included draft protocols for these studies in the submission. Subchronic studies are reviewed below.

3.2.1 Doxycycline hyclate: 14 day oral (gavage) dose range finding study in the rat, study No. CNX/9/96, in-life 11/95, report dated 7/11/96, conducted by in compliance with OECD Good Laboratory Practice regulations.

This study was performed to provide information concerning dosage selection for a 13-week study (reviewed below). Doxycycline hyclate (lot No. MA51TJ0503) was dissolved in water and administered by gavage to groups of 15 Crl:CD (SD) BR rats of each sex in dosages of 0 (vehicle control), 50, 100, 200, and 400mg/kg/day for 14 consecutive days. The dosages used in this study are compared to the proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual) below:

Rat dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)
50	54	10
100	109	19
200	217	38
400	435	76

The parameters that were monitored included mortality, clinical observations, body weight, food consumption, gross pathology, organ weights, and limited toxicokinetic analysis.

Results:

Mortalities. No unscheduled deaths.

Clinical signs. Excessive salivation at 400mg/kg/day.

Body weight. No remarkable effects.

Food consumption. The mean rate of food consumption was reduced by 12% in high-dose males. All other treatment groups were within 6% of controls.

Gross necropsy. No remarkable observations.

Organ weights. A trend toward reduced mean liver weight was observed in treated animals, particularly males.

Conclusion. Under the conditions of this study, administration

of doxycycline was associated with only minor evidence of toxicity at dosages up to and including 400mg/kg/day.

3.2.2 13-week oral (gavage) toxicity study in the rat, study No. CPF00403, in-life 1/96-4/96, report dated 7/11/96, conducted by in compliance with OECD Good Laboratory Practice regulations.

Methods: Approximately 6-week old Hsd:Sprague Dawley rats were randomly assigned into treatment groups as indicated below:

Group	Dose (mg/kg)	Number of Animals Male	Female
Main study Animals			
1 Control	0	10	10
2 Low-dose	25	10	10
3 Mid-dose 1	100	10	10
4 Mid-dose 2	400	10	10
5 High-dose	600	10	10
Toxicokinetic Animals			
6 Control	0	18	18
7 Low-dose	25	18	18
8 Mid-dose 1	100	18	18
9 Mid-dose 2	400	18	18
10 High-dose	600	18	18

The animals were dosed once daily by gavage, 7 days per week for 13 weeks. The dose volume for all animals was 10ml/kg. Water was used as a vehicle. The concentrations of the test solutions were 0, 2.5, 10, 40, and 60mg/mL for control through high-dose groups, respectively. The lot number of the test article (doxycycline hyclate) was MA51TJ0503. The test solutions were protected from light and stored at 4°C for not more than 5 days. Food and water were available *ad libitum*. The dosages used in this study are compared to the maximum proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual; clinical AUC_{0-24} is approx. 11,000 ng·h/mL) below:

Rat dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)	Approx. AUC Ratio
25	27	5	1.3
100	109	19	5
400	435	76	15
600	652	114	14

Main study animals (groups 1-5, above): The parameters that were monitored were survival, clinical signs, body weight, food and water consumption, ophthalmology, hematology, blood

chemistry, urinalysis, gross necropsy, organ weights, and histopathology (groups 1 and 5 only) of the adrenals, aorta, brain, epididymides, esophagus, eyes, heart, kidneys, large intestine, liver, lungs, mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, small intestine, spinal cord, spleen, sternum, stomach, submandibular lymph node, testes, tongue, thymus, thyroid, trachea, urinary bladder, uterus, vagina, and any gross lesions or masses. In addition, "target" tissues in the high-dose animals were examined in the lower groups.

Toxicokinetic animals (groups 6-10, above): Blood samples were collected from three animals per sex from groups 6-10 on days 1 and 91 at 0.5, 1, 2, 4, 8 and 24 hours after dosing. The concentration of doxycycline in the plasma was determined for each sample, and the data were used to calculate the C_{max}, t_{max}, and the AUC_{0-24}.

Results.

1. Toxicology data.

Mortalities. Including animals in the "toxicokinetic" (satellite) group, 4 males and 3 females at 600mg/kg/day, and 1 male at 400mg/kg/day, were found dead or were sacrificed *in extremis* during the study; 2 high-dose males were killed on day 3, 2 high-dose males and 2 high-dose females were killed on day 6, 1 male at 400mg/kg/day was found dead on day 22, and 1 female at 600mg/kg/day was found dead on day 63. Some of the animals that died during the first week were replaced with animals from the original pool. Two males and 2 females in group 5 (high-dose main-study animals) died prematurely, and of these animals, 1 male and both females were judged (post-mortem) to have died as a result of gavage accidents. The cause of death of the remaining male was judged to have been gastric erosion (presumably related to treatment). Since "terminal investigations" were limited to groups 1-5, it is unknown how many of the deaths among the toxicokinetic animals (2 high-dose males, 1 high-dose female, and 1 male at 400mg/kg/day) may have been secondary to gavage accidents.

Clinical signs. Adverse signs were observed only at 400 and 600mg/kg/day, and included labored breathing, subdued behavior, stained fur, salivation, hunched posture, and distended abdomen. The severity and incidence of these signs decreased substantially by week 6.

Body weight. A trend toward reduced mean body weight gain was observed in male animals that received 600mg/kg/day of the test substance. However, a statistically significantly difference in mean weight gain was not observed over weeks 1-14. No trends or effects on weight gain were observed in females.

Food consumption. The mean rate of food consumption was apparently reduced in both males and females at 400mg/kg/day and above during the first week of dosing, but not at later time points. Water consumption (monitored only during the last few weeks of the study) was increased in all animals at 400mg/kg/day and above.

Ophthalmology. No remarkable effects.

Hematology. Males at 400mg/kg/day and above exhibited reductions in RBC count, hemoglobin, hematocrit, and activated partial thromboplastin time. Females at 600mg/kg/day exhibited reductions in RBC count and hemoglobin. Females at all dosages of doxycycline exhibited reduced WBC counts. In general, these effects were minor:

Males:

Dosage (mg/kg /day)	RBC ($x10^6/\mu L$ ±SD)	Hb (g/dL ±SD)	HCT (%±SD)	APTT (sec ±SD)
0	8.77±0.30	15.4±0.4	47.1±1.2	20.6±1.6
25	8.74±0.29	15.4±0.3	46.9±0.8	20.7±1.3
100	8.56±0.43	15.4±0.6	46.4±2.2	19.8±2.2
400	8.09±0.27*	14.6±0.6*	44.4±1.6*	17.7±1.4*
600	8.21±0.55*	14.7±0.9*	45.1±2.7*	18.2±2.0*

* indicates $p<0.05$

Females:

Dosage (mg/kg /day)	RBC ($x10^6/\mu L$ ±SD)	Hb (g/dL ±SD)	WBC ($x10^3/\mu L$ ±SD)
0	7.95±0.31	15.1±0.7	12.6±3.7
25	8.07±0.29	15.3±0.5	9.5±1.5*
100	7.85±0.27	15.0±0.5	9.8±1.4*
400	7.89±0.45	14.8±0.7	9.3±1.5*
600	7.65±0.21	14.5±0.5*	10.1±1.7*

* indicates $p<0.05$

No other remarkable hematological effects were observed.

Blood chemistry. Males at 400mg/kg/day and above exhibited

slight increases in ALP and AST (but no effect on ALT or LDH), slight decreases in total protein and globulin (but no effect on albumin), and slight increases in cholesterol, phosphate, and sodium. Females at 600mg/kg/day exhibited increased urea and reduced triglycerides. Females at all dosages exhibited reductions in total protein and globulin without effect on albumin. In general, these effects were minor:

Males:

Dosage (mg/kg /day)	ALP (U/L ±SD)	AST (U/L ±SD)	Pro-tein (g/L ±SD)	Glob. (g/L ±SD)	Chol. (mmol/ L±SD)	Phos. (mmol/ L±SD)	Na (mmol/ L±SD)
0	137±16	81±8	69±2	38±3	2.6±.2	2.7±.3	142±2
25	136±22	83±10	70±4	39±4	2.6±.4	2.7±.1	142±3
100	132±13	87±9	68±3	36±2	2.7±.3	2.9±.3 *	143±1
400	146±16	90±7*	65±2*	32±2*	2.9±.3 *	3.0±.2 *	146±2*
600	142±25	95±15*	63±3*	32±2*	3.3±.3 *	3.0±.3 *	145±3*

* indicates p<0.05

Females:

Dosage (mg/kg/day)	Urea (mmol/L±SD)	Trig. (mmol/L±SD)	Protein (g/L ±SD)	Glob. (g/L ±SD)
0	7.3±0.8	0.8±0.2	70±4	38±4
25	7.8±0.6	0.7±0.2	67±3*	34±2*
100	7.4±0.6	0.7±0.2	68±1*	34±2*
400	8.0±1.1	0.7±0.3	64±3*	31±2*
600	9.3±1.2*	0.5±0.2*	64±2*	31±2*

* indicates p<0.05

No other remarkable effects on blood chemistry were observed.

Urinalysis. A trend toward increased osmolarity and decreased volume was apparent in males at 400mg/kg/day and above. No other remarkable urological effects were observed.

Gross necropsy. Thyroid glands of males and females at 400mg/kg/day and males only at 100mg/kg/day were observed to be abnormally dark at necropsy. Lesions of the stomach were occasionally observed in animals of both sexes at 600mg/kg/day, and included red foci, reddening, and discoloration or thickening of the mucosa. Distention of the cecum was observed in animals of both sexes at 400mg/kg/day.

Organ weights. Significant differences or trends were observed in the absolute mean weights of several organs in both males and females that received 400mg/kg/day or more of the test substance:

Males:

Dosage (mg/kg/day)	Mean Heart Weight (g±SD)	Mean Kidney Weight (g±SD)	Mean Liver Weight (g±SD)	Mean Spleen Weight (g±SD)
0	1.51±0.17	2.49±0.20	13.98±1.09	0.86±0.10
25	1.48±0.14	2.55±0.25	13.78±1.45	0.83±0.09
100	1.46±0.13	2.54±0.33	14.19±2.34	0.85±0.08
400	1.31±0.12*	2.72±0.14	12.63±1.28	0.74±0.10*
600	1.37±0.11*	2.72±0.29	12.63±1.18	0.66±0.07*

* indicates $p<0.05$

Females:

Dosage (mg/kg/day)	Mean Kidney Weight (g±SD)	Mean Liver Weight (g±SD)	Mean Spleen Weight (g±SD)
0	1.66±0.16	8.48±0.58	0.72±0.08
25	1.62±0.10	8.14±0.31	0.68±0.09
100	1.68±0.17	8.32±0.73	0.68±0.06
400	1.83±0.18*	8.51±0.63	0.62±0.03*
600	1.91±0.25*	9.09±1.05	0.61±0.08*

* indicates $p<0.05$

Similar results were obtained following normalization of the data to body weight.

No other remarkable effects on organ weight were observed in either male or female animals.

Histopathology. Treatment-related effects were observed in the thyroid glands, spleen, stomach, duodenum, and cecum of both sexes, and in the adrenal glands of males. A NOAEL was not observed for the thyroid. The NOAEL for the other organs was 100mg/kg/day, with the possible exception of the stomach in males. Specifically:

Thyroid glands. A brown pigment was present in the follicular epithelium of the thyroid in most animals in all treatment groups. The distribution and severity of the pigmentation were dosage-related. Although a NOAEL was not observed for this effect, it (presumably) would not influence survival in a two-year study.

Spleen. Extramedullary hematopoiesis was reduced in the spleen of animals at 600mg/kg/day and in males at 400mg/kg/day.

Stomach. Mild gastritis was present in animals at 400mg/kg/day and above and (the sponsor believes) in males at 100mg/kg/day. Observations included minimal to moderate submucosal inflammation, intracellular edema, hyperkeratosis, moderate to marked focal erosion, and minimal goblet cell hyperplasia. These findings are summarized below:

Histopathology findings in the Stomach

	Males					Females				
Dose level - mg/kg/day	**0**	**25**	**100**	**400**	**600**	**0**	**25**	**100**	**400**	**600**
number of animals	10	10	10	10	11	10	10	10	10	10
STOMACH, FUNDIC										
number examined	10	10	10	10	11	10	10	10	10	10
Submucosal inflammation										
- minimal	1	3	4	2	4	0	0	5	6	4
- slight	0	0	1	7	5	0	0	0	4	4
- moderate	0	0	0	0	1	0	0	0	0	1
Total	1	3	5	9	10	0	0	5	10	9
Focal Erosion										
- moderate	0	0	0	0	1	0	0	0	0	1
- marked	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	1	0	0	0	0	2
Increase in mucous secreting cells										
- minimal	0	0	1	3	2	0	0	0	5	2
STOMACH, NON-GLANDULAR										
number examined	10	10	10	10	11	10	10	10	10	10
Submucosal inflammation										
- minimal	0	0	0	0	1	0	0	0	0	2
Spongiosis at limiting ridge										
- minimal	0	0	4	6	6	0	0	0	1	4
- slight	0	0	0	1	1	0	0	0	5	2
Total	0	0	4	7	7	0	0	0	6	6
Hyperkeratosis										
- minimal	0	0	0	6	7	0	0	0	7	5
- slight	0	0	0	0	1	0	0	0	0	0
Total	0	0	0	6	8	0	0	0	7	5

Descriptive histopathology summary (cont.):

Duodenum. Minimal to slight mucosal hyperplasia with villus hypertrophy was present in the duodenum of the majority of animals at 400mg/kg/day and above.

Cecum. Minimal to slight diffuse mucosal hyperplasia was present in the cecum of the majority of animals at 400mg/kg/day and above.

Adrenal gland. Increased cortical lipidosis was observed in the adrenal gland of males at 400mg/kg/day and above.

Toxicokinetic evaluation. The maximum plasma concentration (C_{max}) and AUC of doxycycline increased with increasing dosage up to 400mg/kg/day, but actually declined slightly when the dosage was increased to 600mg/kg/day. The data are summarized below:

Summary of Toxicokinetic Data

Rats	Dose (mg/kg/day)	C_{max} (ng/mL)		t_{max} (h)		AUC_{0-24} (ng*h/mL)		Accumulatio Ratio (R_c)
		Day 1	Day 91	Day 1	Day 91	Day 1	Day 91	
Male	25	1735	1628	2.00	1.00	18581	14036	0.755
	100	2831	3518	1.99	1.00	38063	55677	1.46
	400	6232	9006	8.00	4.00	108623	159048	1.46
	600	6405	8316	4.00	1.00	104809	151480	1.45
Female	25	1704	2016	1.99	0.50	17383	13385	0.770
	100	3295	3458	2.00	2.00	36330	53606	1.48
	400	6687	10940	4.01	0.53	108732	173932	1.60
	600	6134	8514	0.99	2.00	114626	152643	1.33

Summary and reviewer's comments concerning the 13-week rat Study: Toxicity was observed at 400mg/kg/day and above, including adverse clinical signs, a trend toward reduced weight gain, suppressed erythrocytic parameters, reduced plasma protein, reduced weight and hematopoietic activity of the spleen, and mild inflammation of the GI tract, including moderate to marked focal erosions of the stomach (observed at 600mg/kg/day only). Mortalities occurred in both males and females at 600mg/kg/day, but at least some of these were secondary to gavage accidents. A dose of 100mg/kg/day was a NOAEL with the exception of causing moderate accumulation of brown pigment in follicular epithelial cells of the thyroid gland and possibly inducing extremely minor

inflammation of the GI tract. Brown pigment was deposited in the thyroid gland of essentially all treated animals (including those in the low-dose group); the distribution and quantity of pigment deposition were related to dosage. The significance of the pigment distribution, if any, is unknown.

3.2.3
will be performed as a phase-4 commitment. Please see section 3.5 of this review for details of the protocol for that study.

3.2.4 Four week oral toxicity study in the cynomolgus monkey, study No. CPF00404, in-life 4/96-5/96, report dated 7/11/96, conducted by in compliance with OECD Good Laboratory Practice regulations.
Methods: Approximately 2-year old captive-bred cynomolgus monkeys were randomly assigned into four treatment groups, each group consisting of three animals per sex. Treatment consisted of administration of doxycycline hyclate at dosages of either 0 (control), 5, 25, and 50mg/kg/day. The animals were dosed once daily by gavage, 7 days per week for 28 days. The dose volume for all animals was 5ml/kg. Water was used as a vehicle. The concentrations of the test solutions were 0, 1, 5, and 10mg/mL for control through high-dose groups, respectively. The lot number of the test article (doxycycline hyclate) was MA51TJ0503. The test solutions were protected from light and stored at 4°C for not more than 3 days. The dosages used in this study are compared to the maximum proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual) below:

Monkey dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)
5	5	2
25	27	10
50	54	19

The parameters that were monitored included survival, clinical signs, body weight, ophthalmology, ECG, hematology, blood chemistry, urinalysis, gross necropsy, organ weights, and histopathology of all major tissues. Blood samples were obtained and analyzed for toxicokinetic evaluation.
Results.
Mortalities. No unscheduled deaths.

Clinical signs. Salivation and vomiting were observed at 25 and 50mg/kg/day; the incidence was proportional to dosage. No adverse signs were observed at 5mg/kg/day.

Body weight. No remarkable effects.

Ophthalmology. No remarkable effects.

ECG. No remarkable effects.

Hematology. No remarkable effects.

Blood chemistry. A trend toward a dosage-related increase in the plasma concentration of urea was apparent in treated animals. No other remarkable effects on blood chemistry were observed.

Urinalysis. No remarkable observations.

Gross necropsy. No remarkable observations.

Organ weights. A trend toward a decrease in mean adrenal weight and an increase in mean kidney weight was observed in high-dose males. No other remarkable effects on organ weight were observed in either male or female animals.

Histopathology. Treatment-related effects were observed in the kidney. Specifically, a dosage-related increase in the incidence and severity of tubular degeneration/regeneration was observed. These lesions occurred mainly in the tubules and ducts in the medullary rays and varied in severity from minimal to moderate. The change exhibited mainly as a swelling of the cytoplasm of the tubular epithelial cells with occasional apoptosis and some loss of nuclear staining. Regenerative hyperplasia of epithelial cells was observed, particularly in more severely affected animals. Tubular degeneration/regeneration was present diffusely in all or most of the medullary rays in some animals and in occasional foci in others. Interstitial edema (primarily in the medulla) was observed in some of the more severely affected animals, possibly suggesting an alteration in tubular function. The incidences of the renal lesions are indicated below:

Histopathology findings in the Kidney

	Males				Females			
Dose level - mg/kg/day	**0**	**5**	**25**	**50**	**0**	**5**	**25**	**50**
number of animals	3	3	3	3	3	3	3	3
Focus of tubular degeneration/regeneration								
- minimal	0	1	1	0	1	1	0	1
- slight	0	1	0	0	0	0	1	0
Total	0	2	1	0	1	1	1	1
Diffuse tubular degeneration/regeneration								
- minimal	0	0	0	0	0	0	1	0
- slight	0	0	1	0	0	0	1	0
- moderate	0	0	1	3	0	0	0	2
Total	0	0	2	3	0	0	2	2

Toxicokinetic evaluation. The following toxicokinetic values were calculated:

Summary Statistics for Doxycycline Toxicokinetic Parameters Following Oral Administration of Doxycycline Hyclate to Cynomolgus Monkeys

Day 1

	Dose	C_{max} (ng/mL)		t_{max} (h)	AUC_{0-24} (ng*h/mL)	
Monkeys	(mg/kg/day)	Mean	SD	Median	Mean	SD
Male	5	2134	803	2.00	19332	7371
	25	2272	793	2.00	32729	16517
	50	7031	1362	4.00	94127	21932
Female	5	1009	357	4.00	12691	2631
	25	4997	2345	4.00	66076	36367
	50	4158	375	8.00	61645	10300

Day 5

	Dose	C_{max} (ng/mL)		t_{max} (h)	AUC_{τ} (ng*h/mL)	
Monkeys	(mg/kg/day)	Mean	SD	Median	Mean	SD
Male	5	2231	1234	2.00	25379	12227
	25	3884	1654	4.00	45065	19719
	50	11100	8215	4.00	102947	46807
Female	5	2426	1568	4.00	22403	8983
	25	5431	3891	4.00	62377	32697
	50	5786	2129	2.00	73265	29979

Day 28

	Dose	C_{max} (ng/mL)		t_{max} (h)	AUC_{τ} (ng*h/mL)	
Monkeys	(mg/kg/day)	Mean	SD	Median	Mean	SD
Male	5	995	228	2.00	10058	2018
	25	3415	1371	0.50	37432	14848
	50	4355	34.2	1.01	50545	13129
Female	5	1474	296	2.00	13141	2903
	25	3789	1322	2.00	48139	33590
	50	3736	1096	1.99	52002	20957

Summary and reviewer's comments concerning the 4-week monkey

Study: Administration of doxycycline hyclate to cynomolgus monkeys at doses of 5, 25 or 50 mg/kg/day was generally well tolerated and produced minimal signs of toxicity. Salivation during or immediately after dosing was seen in nine animals from the 25 and 50 mg/kg/day groups. No treatment related changes in hematology or urinalysis were seen. Histopathological examination revealed a dosage-related increase in incidence and severity of tubular degeneration/regeneration in the kidney, which may correlate with a trend toward increased plasma urea that was observed in mid and high-dose animals.

3.2.5

will be performed as a phase-4 commitment. A protocol for this study was included in the submission and is briefly reviewed below.

One year oral (gavage) toxicity study in the cynomolgus monkey, to be conducted by

in compliance with OECD Good Laboratory Practice regulations.

In the proposed study, approximately 2-year old captive-bred cynomolgus monkeys would be randomly assigned into four treatment groups, each group consisting of four animals per sex. Treatment would consist of administration of doxycycline hyclate at dosages of either 0 (control), 1, 4, or 16mg/kg/day. The proposed dosages are compared to the maximum proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual; clinical AUC_{0-24} is approx. 11,000 ng·h/mL) below:

Monkey dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)	Approx. AUC Ratio
1	1	0.38	0.4
4	4	1.5	0.7
16	17	6	2

The animals would be housed by sex and dose (groups of four). The animals would be dosed once daily by gavage (5ml/kg), 7 days per week, for 52 weeks. Water would be used as a vehicle. The test solutions would be stored in the dark at 4°C and used within 5 days of preparation. The protocol indicates the test substance has been shown to be adequately stable in aqueous solution for 8 days when stored in the dark at 4°C. The parameters that would be monitored include survival, clinical signs, body weight, feeding patterns, ophthalmology, complete hematology (including WBC differential count), blood chemistry, urinalysis, gross necropsy, organ weights, and histopathology of all major tissues. Blood samples would be obtained at five (currently unspecified) time points from all animals on day 1 and during weeks 25 and 52 of treatment. The sponsor will be asked to clarify this matter.

Reviewer's comments concerning the draft protocol for the chronic monkey study: [Note: Similar comments have been FAXed to the sponsor; see memorandum to NDA 50-744 dated 10/21/96]. I consider the proposed dosages to be too low. The low dose and mid dose would both result in lower levels of exposure to the test material than would be achieved clinically (AUC ratios less than one), while the duration of exposure (one year) is the same as the proposed duration of clinical use. Even the high-dose would yield an AUC ratio of only two. Such data would be of little value. Given that relatively little toxicity was observed at dosages up to 50mg/kg/day (the highest dosage examined) in the four-week dosage selection study, I recommend that dosages of 5, 15, and 45mg/kg/day be utilized. This should result in AUC ratios of approximately 1, 2, and 4, respectively. Even higher levels of exposure would be desirable, but may not be compatible with survival in a one-year study. The T_{max} at steady state observed in the four-week study occurred at approximately two hours post-dosing. I recommend that 0.5, 1, 2, 4 and 6 hours post-dosing be selected as the five time points at which blood samples will be obtained for toxicokinetic analysis. I consider the protocol to be acceptable in other respects.

3.3 Reproductive Toxicology.
As noted on page two of this review, it was agreed in a meeting with the sponsor on 12/21/95 that teratology studies could be waived because pregnancy category "D" class labeling is associated with tetracyclines.

3.3.1. Oral (gavage) rat fertility and general reproductive performance study, Study No. CNX/2/96, report dated 7/11/96, conducted by in compliance with Good Laboratory Practice regulations (21 CFR 58).

This study was conducted to assess the toxicity potential of doxycycline hyclate on the fertility and reproductive performance of male and female rats when administered orally once daily. Sprague-Dawley OFA(SD)IOPS-Caw rats were obtained from Treatment consisted of administration of doxycycline hyclate at dosages of either 0 (control), 50, 100, 250, and 500mg/kg/day. The animals were dosed once daily by gavage; males were treated for at least 28 days prior to mating and throughout the mating period, while females were treated beginning 14 days prior to pairing and continuing until day 7 of pregnancy. The dose volume for all animals was 10ml/kg. Water was used as a vehicle. Food and water were available *ad libitum*. The dosages used in this study are compared to the maximum proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual) below:

Rat dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)
50	54	10
100	109	19
250	272	48
500	543	95

Following at least 28 days treatment for males and 14 days treatment for females, 1 male and 1 female from the same dosage group were co-habitated for up to 7 days. Mating was confirmed through daily vaginal smears. If a pair failed to mate within 7 days, the male was replaced with a male from the same treatment group that had mated previously and the new pair was monitored for mating for up to 10 days. Males from the original pairs that had failed to mate were repaired with untreated females and monitored for mating for up to 10 days. Following completion of the first mating period (including re-pairings), after 46 days of treatment, all males in the control, 250, and 500mg/kg/day groups were paired with virgin, untreated females (apparently for 7 days). Females were killed on day 13 of pregnancy. In females, the parameters that were monitored included the pregnancy status and the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead embryos. In males, the testes were weighed and sectioned (both plastic and paraffin

sections); only sections from control and high-dose animals were microscopically examined. The epididymides were removed and sperm samples were collected from them. The sperm samples were examined for concentration, motility, and morphology.

Results.

Clinical signs and survival: Two males at 500mg/kg/day were sacrificed early in the study (days 2 and 4) due to poor clinical signs (piloerection, hunched posture, salivation, labored breathing). Clinical signs observed in other animals at 500mg/kg/day included soft stools, fur staining, salivation, and labored breathing. Similar signs were observed at 100 and 250mg/kg/day, although the incidence and severity appeared to be proportional to the dosage. No treatment-related signs were observed at 50mg/kg/day.

Body weight:

Males: At 500mg/kg/day, the mean body weight was significantly decreased relative to controls at most time points during the treatment period. There appeared to be no remarkable effects on body weight in males at 250mg/kg/day and below.

Females: No remarkable effects.

Fertility and mating performance: The mean time required for mating to occur following pairing of treated males and females tended to increase with increasing dosage; the mean numbers of days for mating to occur were 2.6, 2.8, 3.5, 3.1, and 4.0 in the 0, 50, 100, 250, and 500mg/kg/day groups, respectively. However, statistical difference was not achieved. The numbers of females that failed to mate within the first estrous cycle in these groups were 0, 1, 2, 1, and 4, respectively. No treatment-related effects on the fertility index (percentage of mated females that became pregnant) were apparent following the initial pairing. During the second mating period (control, 250, and 500mg/kg/day males paired with untreated females), all animals copulated within the first estrous cycle, but only 15 of 23 males at 500mg/kg/day succeeded in impregnating an untreated female, yielding a fertility index of 65%, which was considered to be significantly less than the fertility index of control animals (96%). However, in the absence of an effect on fertility during the initial mating period (when both males and females were treated), it is unclear what, if anything, this decrease in the fertility index meant. Possibly, a decrease in fertility became apparent after 46 days of treatment, but not after 28 days of treatment.

Testes weight: No remarkable effects.

Sperm analysis: A dosage-related, statistically significant reduction in straight-line velocity of sperm was observed at all

treatment levels. Males at 500mg/kg/day exhibited a statistically significant increase in sperm with abnormal morphology, including sperm that were missing either the head or the tail, as well as significant decreases in the percentage of sperm that were motile and in the number of sperm per unit volume. Animals at 100 and 250mg/kg/day exhibited non-significant trends toward increased numbers of morphologically abnormal sperm. Histological examination of sections of the testes produced no remarkable findings.

Observations made following cesarean section:
Treated females. At 500mg/kg/day, the mean numbers of corpora lutea, implantations, and live embryos were statistically significantly reduced, and pre and post-implantation losses were increased relative to controls. At 250mg/kg/day, the mean number of implantations was reduced, resulting in an increased pre-implantation loss. No other remarkable effects were observed.

Untreated females (paired with treated males). Untreated females that were paired with males that received either 250 or 500mg/kg/day exhibited a statistically significant increase in the pre-implantation loss and a non-significant trend toward an increase in the post-implantation loss.

Summary/conclusions: Under the conditions of this study, oral administration of doxycycline at sufficient dosage adversely affects fertility and reproductive performance of rats, as evidenced by increased time for mating to occur, reduced sperm motility, velocity, and concentration, abnormal sperm morphology, and increased pre- and post-implantation losses. A NOAEL was not demonstrated in this study, since even the lowest dosage tested (50mg/kg/day) induced a statistically significant reduction in sperm velocity.

3.3.2. Oral (gavage) rat developmental (pre- and post-natal) toxicity study, Study No. CNX/3/96, report dated 7/11/96, conducted by in compliance with Good Laboratory Practice regulations (21 CFR 58).

This study was conducted to assess the toxicity potential of doxycycline hyclate on parturition, lactation, and pup development following oral administration to pregnant rats during the latter phase of pregnancy and throughout lactation. Sprague-Dawley OFA(SD)IOPS-Caw rats were obtained from F0 females were paired overnight with a mature male (2 females to 1 male). Vaginal smears were taken each morning; the day on which sperm were observed was designated day 0 of pregnancy. Treatment consisted of administration of doxycycline hyclate at dosages of either 0 (control), 50, 100, 250, and 500mg/kg/day. Pregnant F0 females were dosed once daily by

gavage from day 18 of pregnancy through day 20 postpartum (weaning); males were not treated. The dose volume for all treated animals was 10ml/kg. Water was used as a vehicle. Food and water were available *ad libitum*. The dosages used in this study are compared to the maximum proposed clinical dosage (46mg/day, or 0.92mg/kg/day in a 50kg individual) below:

Rat dose (mg/kg/day)	Multiple of human dose (mg/kg)	Multiple of human dose (mg/m^2)
50	54	10
100	109	19
250	272	48
500	543	95

A. F0 generation:
The F0 females were monitored for survival, clinical signs, bodyweight, and food consumption. The F0 females were examined during parturition for evidence of difficult or abnormal delivery. Litter size and sex ratio were monitored daily. The F0 rats were sacrificed on day 21 of lactation and subjected to necropsy, at which time the number of implantation scars in the uterus was recorded.

B. F1 generation:
All pups were examined for malformations as soon after birth as possible. The pups were examined daily for signs of toxicity. On day 4, the litters were culled (randomly) to 4 pups per sex. Individual pup weights were obtained on days 0, 4, 7, 14, and 21 postpartum. Developmental, functional, and behavioral evaluations were performed on F1 rats, including pinnae detachment, righting reflex, eye opening, pupillary light reflex, startle response, and auditory response. Following weaning, 20 F1 animals per sex per group were selected for assessment of learning potential in a water maze. Necropsy was performed on all F1 animals.

Results.

A. F0 generation:

Survival and clinical signs: No treatment-related deaths occurred among F0 animals. F0 females at 500mg/kg/day exhibited noisy breathing and diarrhea during the treatment period.

Body weight and weight gain: No significant effects on mean bodyweight. Mean maternal body weight gains were reduced at 500mg/kg/day over days 1 through 4 postpartum, and increased at subsequent time points. No treatment-related effects on body weight were observed at lower dosages.

Food consumption: The mean rate of food consumption was statistically significantly reduced in all treatment groups

relative to controls on days 18 through 20 of pregnancy, and in high-dose F0 animals over days 1 through 7 postpartum.

Pregnancy and litter data: No meaningful differences in gestation length, litter size, or pup survival were apparent.

Necropsy: No treatment-induced lesions were observed in any F0 rats during necropsy.

B. F1 generation:
Clinical signs: The number of litters with hair loss, piloerection, or abnormal hair growth increased in proportion to dosage.

Mean pup weight and weight gain: Trends toward reductions in mean weight and mean weight gain of F1 animals were apparent in the high-dose group during the period of lactation. No significant differences were observed at the other dosage levels.

Developmental landmarks and functional testing: No significant effects were observed on ear opening, righting reflex, or eye opening. F1 animals in the high-dose group exhibited statistically significant reductions in the percentages of animals that exhibited a startle response on day 15 and a pupillary light reflex on day 21. However, the percent reductions were small (97.7% and 96.5%, respectively, versus control responses of 100%).

Behavior, learning and memory ability: No remarkable findings.

Growth and development after weaning: No treatment-related differences.

Necropsy: No remarkable observations were made during necropsy.

Conclusions: Under the conditions of this study, doxycycline did not induce substantial effects on parturition, lactation, or pup development. Adverse clinical signs, trends toward reduced body weight gain, and reduced food consumption were observed in F0 females at high doses of the test substance, and offspring of animals that received 100mg/kg/day or more exhibited increased hair loss, suggesting they were under stress, but no effects on pup survival, development, or learning ability were seen. It seems unlikely that effects on parturition, lactation, or development would occur in human mothers or their infants as a result of use of Periostat. Periostat would, however, be contraindicated in pregnant or nursing women (as are all tetracyclines) because of the possibility of inducing discoloration of the teeth of the infant.

3.4 Genetic Toxicology.
As noted on page two of this review, it was agreed in a meeting with the sponsor on 12/21/95 that it would be inappropriate to conduct an Ames test with a tetracycline. A mammalian point mutation assay (CHO HGPRT) was requested in lieu of an Ames test.

3.4.1 Point mutation in Chinese hamster ovary (CHO) cells, study No. M/PMC/41387, in-life 6/95, conducted by in compliance with Good Laboratory Practice regulations (21 CFR 58).

CHO cells, obtained from the European Collection of Animal Cell Cultures, were incubated for 3 hours at 37°C (with and without S9) in culture medium to which doxycycline hyclate (lot No. MA51TJ0503) or a positive or negative control agent had been added; concentrations of doxycycline tested ranged from µg/mL. Appropriate positive controls were used. Following the exposure period, the cells were processed, re-plated and incubated during a 7 day "expression" period, and then reprocessed and grown for 7 days in hypoxanthine-free medium that contained 24µM 6-thioguanine. The cultures were then fixed and the numbers of colonies were counted.

Results: No significant change in the rate of cell survival in the presence of 6-thioguanine (gene mutation) was observed at any concentration of the test material that was used, either with or without metabolic activation. Significant responses were produced by the positive control substances.

Conclusion: These data suggest that the test material is not a genetic toxicant.

3.4.2 In vitro mammalian cell cytogenetic test in CHO cells, study No. M/CCA/41363, in-life 6/95, conducted by in compliance with Good Laboratory Practice regulations (21 CFR 58).

Doxycycline hyclate (lot No. MA51TJ0503) was assayed for the ability to induce chromosomal aberrations in cultured CHO cells, both in the presence and in the absence of metabolic activation (S9). The cells were exposed to doxycycline in concentrations ranging up to 350µg/mL according to standard procedures. Water (the solvent) was used as a negative control; cyclophosphamide was used as a positive control in experiments with S9, and mitomycin C was used as a positive control in experiments without S9. All cultures were treated with colchicine prior to harvest. Prepared slides were examined for chromosomal aberrations.

Results. Exposure to doxycycline significantly increased the percentage of cells that exhibited chromosomal aberrations in some of the assays, although the increases were not consistent or related to dosage. The positive control compounds significantly increased the incidence of chromosomal aberrations.

Conclusions. Doxycycline hyclate was a weak clastogen under the

conditions of this study.

3.4.3 Mouse micronucleus test, study No. M/MMN/41362, in-life 6/95, conducted by
in compliance with Good Laboratory Practice regulations (21 CFR 58).

Doxycycline hyclate (lot No. MA51TJ0503) was assessed for effect on the incidence of micronucleated polychromatic erythrocytes in CD-1 mice. The animals each received a single PO dose of either doxycycline (500, 800, or 1250mg/kg; 15 males, 15 females), water (negative control; 15 males, 15 females), or (by IV injection) 4mg/kg mitomycin C (positive control; 5 males, 5 females). Bone marrow smears were obtained from water and doxycycline-treated mice (5/sex) at 24, 48, and 72 hrs. post-dosing; positive controls were sacrificed at 24 hrs. only. The smears were processed and examined for the number of micronucleated cells per 1000 polychromatic cells examined.

Results. No statistically significant differences were observed between the test substance and the negative control. A significant increase in the occurrence of micronucleated cells relative to the number of polychromatic erythrocytes was observed in smears from positive control animals. Two females that received 1250mg/kg doxycycline died prematurely.

Conclusions. These data provide no evidence that the test substance is clastogenic.

3.5 Carcinogenicity.

As noted on page two of this review, it was agreed in a meeting on 12/21/95 that carcinogenicity data may be limited to one species, The sponsor has committed to performance of for this study in the submission. The protocol has been reviewed and presented to the exec-CAC; the review and conclusions of the division and the exec-CAC are presented below.

Review of the Draft Protocol for the Bioassay:

Two year oral (gavage) carcinogenicity/toxicity study with 6 month interim kill in the rat, to be conducted by

in compliance with OECD Good Laboratory Practice regulations.

The proposed study would utilize HSD:Sprague Dawley rats. The animals would be examined for signs of poor health upon receipt. Shortly after arrival, 10 animals of each sex would be selected for a "baseline health screen", which would consist of "limited" hematological examination (specific parameters not indicated) and histopathological examination of the heart, ileum, lungs, liver, and kidneys. The remaining animals would then be randomly assigned into treatment groups as indicated below:

Group	Proposed Dose (mg/kg)	Number of Animals Male	Female
Carcinogenicity Animals			
1 (Control group 1)	0	60	60
2 (Control group 2)	0	60	60
3 Low-dose	10	60	60
4 Mid-dose	20	60	60
5 High-dose	60	60	60
Chronic Toxicology Animals			
6 Control	0	20	20
7 Low-dose	10	20	20
8 Mid-dose	20	20	20
9 High-dose	60	20	20

At initiation of treatment (following an acclimation period of 6 to 9 days) the animals would be not more than 42 days old. The animals would be housed by sex in groups of five. A commercial rodent feed and water would be available *ad libitum*. The animals would be dosed once daily by gavage (10ml/kg), 7 days per week, for 104 weeks (groups 1-5) or 26 weeks (groups 6-9). Purified water would be used as a vehicle. The test solutions would be stored in the dark at 4°C and used within 5 days of preparation. The protocol indicates the test substance has been shown to be

adequately stable in aqueous solution for 8 days when stored in the dark at 4°C.

Observations:
Carcinogenicity Animals (Groups 1-5). The animals would be examined daily for clinical signs, twice daily for mortality and moribundity, and at least once weekly for palpable masses. Additional parameters that would be monitored include body weight (weekly), gross necropsy, and histopathology (all groups). Tissues to be subjected to histopathology would include:

Adrenals	Mesen. Lymph Node	Stomach
Aorta	Ovaries	Submandib. Lymph Node
Brain	Pancreas	Testes
Cecum	Pituitary	Thymus
Colon	Prostate	Thyroids
Duodenum	Rectum	Tongue
Epididymides	Salivary Gland	Trachea
Esophagus	Sciatic Nerve	Urinary Bladder
Eyes	Seminal Vesicles	Uterus
Heart	Site of Mammary Gland	Vagina
Ileum	Skeletal Muscle	
Jejunum	Skin	
Kidneys	Spinal Cord	
Liver	Spleen	
Lungs	Sternum (incl. marrow)	

And all gross lesions.
Note: See comment 2, on page 28, for recommended additions to this list.

Chronic Toxicology Animals (Groups 6-9). The animals would be examined daily for clinical signs and twice daily for mortality and moribundity. Additional parameters that would be monitored include body weight (weekly), food consumption (per cage), ophthalmology, complete hematology (including WBC differential count), blood chemistry, urinalysis, gross necropsy, organ weights, and histopathology of all tissues listed above from animals in groups 6 and 9. For the low and mid-dose animals (groups 7 and 8), only those tissues that had been identified as "target tissues" (treatment-affected tissues) in group 9 animals would be histologically examined. The protocol states that "blood and urine samples will be obtained from 10 animals in groups 6-9 during week 13 and 24 of treatment"; presumably, this means that 10 animals *per sex* per group would be sampled after 13 weeks and the remainder of the animals would be sampled after 24 weeks, and that the animals would be properly randomized into either the 13 week or the 24 week sampling groups. The sponsor will be asked to clarify this matter.

Toxicokinetics. The draft protocol indicates that blood samples

would be obtained for determination of the plasma concentration of the test article. As proposed, two animals per sex from groups 6 through 9 would be sampled on the first day of dosing and during the third and sixth month of dosing at five, currently unspecified, time points after treatment. In addition, blood samples would be collected from two animals per sex from groups 1 through 5 immediately prior to scheduled sacrifice, again at five currently unspecified time points after treatment. Sampling would consist of collection of 1ml of blood through the orbital sinus. Samples collected from control animals would not analyzed.

Recommended Modifications to the Protocol:
The following changes in the protocol were suggested by the division and/or the exec-CAC; these recommendations have been made to the sponsor:

1. It is recommended that the study utilize dosages of the test substance of 0, 20, 75, and 200mg/kg/day.

2. It is recommended that the list of tissues that would be histologically examined be expanded to include the Harderian gland, lacrimal gland, larynx, bronchi, nasopharynx, parathyroid gland, and Zymbal's gland.

3. It is recommended that the protocol be modified to specify that bone marrow smears from animals in groups 1 through 5 will be microscopically examined.

4. It is recommended that the protocol be modified such that (in lieu of the proposed toxicokinetic evaluation): 1) blood samples be obtained for the purpose of toxicokinetic assessment only from animals in groups 6 through 9 and only after six months on study; 2) toxicokinetic blood samples be obtained from 3 animals per sex per group (groups 6 through 9 only) immediately before dosing (time "0") and at 0.5, 1, 2, 4, and 12 hours after dosing. The benefits of this approach include the facts that toxicokinetic data would be obtained from 3 animals per sex per group per time point instead of 2 animals, and it would not be necessary to bleed the "chronic toxicity" animals prior to sacrifice.

5. Section 10.0 of the protocol states that "Blood and urine samples will be obtained from 10 animals in groups 6-9 during week 13 and 24 of treatment". It is recommended that this be modified to state that clinical pathology will be performed on blood and urine samples obtained from 10 randomly selected animals per sex from groups 6-9 after six months of treatment, and that the blood samples be obtained on the day of scheduled sacrifice.

The recommended study design is summarized below:

Group	Proposed Dose (mg/kg)	Number of Animals Male	Number of Animals Female
Carcinogenicity Animals			
1 (Control group 1)	0	60	60
2 (Control group 2)	0	60	60
3 Low-dose	20	60	60
4 Mid-dose	75	60	60
5 High-dose	200	60	60
Chronic Toxicology Animals			
6 Control	0	20	20
7 Low-dose	20	20	20
8 Mid-dose	75	20	20
9 High-dose	200	20	20

Summary:

Pharmacology. Within the context of this application, doxycycline is believed to act through inhibition of collagenase, preventing the degradation of connective tissue that is associated with periodontitis. Doxycycline does not exert antibacterial effects at the dosage at which it is proposed for marketing under this NDA (20mg twice daily).

Pharmacokinetics. Doxycycline is generally well absorbed from the GI tract, although aluminum-containing antacids will interfere with absorption. Apparently, the extent of protein binding is variable, ranging from %. Published values for the serum half-life and renal clearance of doxycycline are 14.5-22 hours and 16ml/min., respectively. The primary route of elimination of doxycycline is excretion in the bile; elimination of doxycycline is apparently not compromised by renal dysfunction.

Acute toxicology. Doxycycline hyclate did not elicit signs of toxicity when administered to Crl:CD(SD)BR rats by gavage on a single occasion at a dosage of 500mg/kg. Of two males and two females that received single doses of 750mg/kg, only one death occurred (a male).

Multiple-dose toxicology:

Rats. In a 13-week study in which doxycycline hyclate was administered to rats at dosages of 25, 100, 400, and 600mg/kg/day, toxicity was observed at 400mg/kg/day and above, including adverse clinical signs, a trend toward reduced weight gain, suppressed erythrocytic parameters, reduced plasma protein, reduced weight and hematopoietic activity of the spleen, and mild inflammation of the GI tract, including moderate to marked focal erosions of the stomach (the latter effect observed at 600mg/kg/day only). Mortalities occurred in both males and females at 600mg/kg/day, but at least some of these were secondary to gavage accidents. A dose of 100mg/kg/day was a no-adverse-effect-level (NOAEL), with the exception of causing moderate accumulation of brown pigment in follicular epithelial cells of the thyroid gland and possibly inducing extremely minor inflammation of the GI tract. Brown pigment was deposited in the thyroid gland of essentially all treated animals (including those in the lowest-dose group (25mg/kg/day)); the distribution and quantity of pigment deposition were related to dosage. The significance of the pigment distribution, if any, is unknown. For further details, see section 3.2.2 of this review.

Monkeys. Daily administration of doxycycline hyclate to cynomolgus monkeys at dosages of 5, 25 or 50mg/kg/day was

generally well tolerated and produced minimal signs of toxicity. Salivation during or immediately after dosing was seen in nine animals from the 25 and 50mg/kg/day groups. No treatment related changes in hematology or urinalysis were seen. Histopathological examination revealed a dosage related increase in the incidence and severity of tubular degeneration/regeneration in the kidney, which may correlate with a trend toward increased plasma urea that was observed in mid and high-dose animals. For details, see section 3.2.4 of this review.

Reproductive Toxicology:

Fertility/reproductive success. When administered to male and female rats for appropriate periods prior to pairing and throughout mating at dosages of 50 to 500mg/kg/day, doxycycline hyclate adversely affected fertility and reproductive performance of rats, as evidenced by increased time for mating to occur, reduced sperm motility, velocity, and concentration, abnormal sperm morphology, and increased pre- and post-implantation losses. A NOAEL was not demonstrated in the study, since even the lowest dosage tested (50mg/kg/day) induced a statistically significant reduction in sperm velocity. For further details, see section 3.3.1 of this review.

Teratology/fetal toxicity. Teratology studies were waived because pregnancy category "D" class labeling is associated with tetracyclines.

Developmental toxicity (peri/post-natal assessment). When administered to female rats from day 18 of pregnancy through day 20 postpartum at dosages of 50 to 500mg/kg/day, doxycycline did not induce substantial effects on parturition, lactation, or pup development. Adverse clinical signs, trends toward reduced body weight gain, and reduced food consumption were observed in F0 females at high dosages of the test substance, but no effects on pup survival, development, or learning ability were seen. For further details, see section 3.3.2 of this review.

Genetic Toxicology: The genetic toxicology of doxycycline hyclate has been assessed in an *in vitro* point mutation study in mammalian cells (HGPRT in CHO cells), an *in vitro* assay in mammalian cells for potential to cause chromosomal aberrations, and a micronucleus study. The point mutation study and the micronucleus study yielded negative results. The results of the study for potential to cause chromosomal aberrations were equivocal; exposure to doxycycline significantly increased the percentage of cells that exhibited chromosomal aberrations in some of the assays, but the increases were not consistent or related to dosage. The data support a hypothesis that doxycycline is a weak clastogen, but not a mutagen. For further details, see section 3.4 of this review.

Carcinogenicity: The sponsor has committed to

This study will be regarded as a phase-4 commitment.

Labeling: The following modifications of the draft labeling of NDA 50-744 are recommended:

Evaluation: Please see page 30 of this document for a summary of the pharmacology and toxicology of doxycycline. Doxycycline caused little toxicity in the rat at dosages within two orders of magnitude of the proposed clinical dose (100mg/kg/day or less). Substantial toxicity was observed in rats that received higher dosages (400 to 600mg/kg/day), but it is doubtful that these observations would be relevant to clinical use of Periostat. Administration of doxycycline hyclate to cynomolgus monkeys at dosages of 5, 25 or 50mg/kg/day was generally well tolerated and produced minimal signs of toxicity. An increase in the incidence and severity of renal tubular degeneration/regeneration was observed, particularly in monkeys that received 25mg/kg/day or more. However, doxycycline has been used successfully in patients with partial renal failure, and it seems unlikely that the level of exposure to doxycycline hyclate proposed under NDA 50-744 (0.92mg/kg/day in a 50kg individual) would result in renal toxicity. Doxycycline adversely affected reproductive parameters of rats, including causing reduced sperm motility, velocity, and concentration, abnormal sperm morphology, and increased pre- and post-implantation losses. In general, humans are considered to be more sensitive to disruptions of reproductive function than are rats, and the rat fertility data should be interpreted to suggest that humans that are attempting to conceive may wish to abstain from taking Periostat. A comment to this effect has been added to the draft label (see above). In genetic toxicology studies conducted with doxycycline, negative results were obtained in an *in vitro* point mutation study assay) and in an *in vivo* clastogenicity assay (micronucleus assay). However, an *in vitro* clastogenicity assay yielded weakly positive results. The sponsor has agreed to evaluate doxycycline in several chronic toxicology studies as post-approval commitments, including a carcinogenicity bioassay in rats and chronic studies in rats and monkeys. In view of the database accumulated during 30 years of human use of doxycycline, the existing nonclinical data are adequate to support the safety of NDA 50-744. The label of the product will be updated as necessary when data from the "phase-4" studies become available.

Recommendations: NDA 50-744 is approvable in regard to pharmacologic and toxicologic concerns. Recommended changes in the product label are indicated above.

/S/

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Reviewing Pharmacologist

cc:
NDA 50-744
HFD-540 Div. File
HFD-540/TL/JACOBS
HFD-540/PHARM/SEE
HFD-540/MO/GILKES
HFD-540/CHEM/VIDRA
HFD-540/CSO/BLATT
HFD-345

Concurrence Only:
HFD-540/DD/WILKIN 1.14.97
HFD-540/TL/JACOBS 1.9.14.96