

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

50-808

PHARMACOLOGY REVIEW



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 50-808
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 08-JUL-05
PRODUCT: Solodyn 
INTENDED CLINICAL POPULATION: Patients with acne vulgaris
SPONSOR: Medicis Pharmaceutical Corporation
DOCUMENTS REVIEWED: All
REVIEW DIVISION: Division of Dermatology and Dental Products
(HFD-540)
PHARM/TOX REVIEWER: Norman A. See, Ph.D.
PHARM/TOX SUPERVISOR: Paul Brown, Ph.D.
DIVISION DIRECTOR (Acting): Stanka Kukich, M.D.
PROJECT MANAGER: Felecia Curtis

Date of review submission to Division File System (DFS): 22-MAR-06

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: The product is approvable with respect to nonclinical concerns.

B. Recommendation for nonclinical studies: The sponsor has committed to conduct the following nonclinical studies post-approval of the NDA:

1. Evaluation of the carcinogenicity of minocycline HCl in mice. The sponsor should submit a detailed protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 50-808.

2. Evaluation of the carcinogenicity of minocycline HCl in rats. The sponsor should submit a detailed protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 50-808.

C. Recommendations on labeling: It is recommended that the "Carcinogenesis" and "Pregnancy" sections of the label be modified as indicated below:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:

Long-term animal studies have not been performed to evaluate the carcinogenic potential of minocycline. A structurally related compound, oxytetracycline, was found to produce adrenal and pituitary tumors in rats.

Minocycline was not mutagenic *in vitro* in a bacterial reverse mutation assay (Ames test) or in a CHO/HGPRT mammalian cell assay in the presence or absence of metabolic activation. Minocycline was not clastogenic *in vitro* in human peripheral blood lymphocytes or *in vivo* in a mouse micronucleus test.

Male and female reproductive performance in rats was unaffected by oral doses of minocycline of up to 300 mg/kg/day (which resulted in up to approximately 40 times the level of systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets). However, oral administration of 100 or 300 mg/kg/day of minocycline to male rats (resulting in approximately 15 to 40 times the level of systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets) adversely affected spermatogenesis. Effects observed at 300 mg/kg/day included a reduced number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and (at both 100 and 300 mg/kg/day) increased numbers of morphologically abnormal sperm cells. Morphological abnormalities observed in sperm samples included absent heads, misshapen heads, and abnormal flagella. SOLODYN[®] tablets should not be used by individuals who are attempting to conceive a child.

Pregnancy:

Teratogenic Effects: Pregnancy Category D. See **WARNINGS.** Tetracycline-class antibiotics, such as minocycline, are known to be capable of inducing harm to a fetus when administered to a pregnant woman. Minocycline induced skeletal malformations (bent limb bones) in fetuses when administered to pregnant rats and rabbits in doses of 30 mg/kg/day and 100 mg/kg/day, respectively (resulting in approximately 3 times and 2 times, respectively, the systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets). Reduced mean fetal body weight was observed in studies in which minocycline was administered to pregnant rats at a dose of 10 mg/kg/day (which resulted in approximately the same level of systemic exposure to minocycline as that observed in patients who use SOLODYN[®] tablets). SOLODYN[®] tablets should not be used during pregnancy.

II. Summary of nonclinical findings**A. Brief overview of nonclinical findings:**

Minocycline was reasonably well tolerated in general toxicology studies. In 90 day repeat-dose toxicology studies conducted in mice, rats, and monkeys, little toxicity was observed at exposures comparable to the clinical level of exposure. The primary toxicological target organ for minocycline in all species appears to be the thyroid, with treatment-related effects on the thyroid manifesting (at sufficient levels of exposure) as grossly visible enlargement, increased mean weight, dark red discoloration, increased colloid content, accumulation of brown pigment in the follicular cells, follicular cell hypertrophy, and elevated plasma levels of T4 and TSH. These effects are of a minimal to mild severity in most instances, and do not appear to be toxicologically relevant at levels of exposure close to those observed clinically. Minocycline was negative in a battery of genetic toxicology studies, and is apparently not genotoxic. Minocycline may be capable of impairing fertility in human males. When administered to male rats minocycline adversely affected spermatogenic endpoints, including a significant reduction in the mean number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and increased numbers of morphologically abnormal sperm cells. Morphological abnormalities observed in sperm samples included absent heads, misshapen heads, and abnormal flagella. However, male and female reproductive performances were not impaired in that study; there were no effects on the percentages of animals that copulated or became pregnant, or latency to mating. When assessed for teratogenic effects in rats, minocycline reduced the mean fetal weight and induced skeletal malformations (bent limb

bones) and skeletal variations (reduced skeletal ossification). When administered to female rabbits during the period of organogenesis, minocycline induced abortion in a minority of does, reduced maternal weight gain, gravid uterine weight, and mean fetal body weight, and induced skeletal malformations (bent limb bones). When assessed for effects on pre- and postnatal development, including maternal function, in rats, minocycline had no effect on the duration of the gestation period or on the number of dams with all pups dying postpartum. There was no effect on the number of viable pups per litter, although postnatal survival was decreased with increasing exposure to minocycline. Gross external anomalies observed in F1 pups included smallness of size, malrotated forelimbs, and micromelia. No effects were observed on the physical development (time to balanopreputial separation or vaginal opening), behavior, learning ability, or reproduction of F1 pups, and there was no effect on gross appearance of F2 pups. Minocycline was evaluated in a battery of safety pharmacology studies. Minocycline had no effect on behavior, psychological state, arterial blood pressure, heart rate, the ECG, or (at clinically relevant levels of exposure) respiration.

Please see section 2.6.7 of this review for a tabulated summary of the "safety factors" (AUC ratios at the NOAEL) that were demonstrated in the studies mentioned above.

- B. Pharmacologic activity: Minocycline inhibits the growth of certain species of bacteria through inhibition of protein synthesis by blocking aminoacyl-t-RNA binding to the m-RNA-ribosome complex. Minocycline is active against a number of gram-positive and gram-negative organisms, including *Propionibacterium acnes*. The mechanism through which minocycline ameliorates acne is not fully elucidated, although reducing the bacterial count may reduce the size and quantity of lesions by reducing inflammation.
- C. Nonclinical safety issues relevant to clinical use: The label of the product should describe the reproductive toxicology of minocycline, as summarized above. Minocycline impaired spermatogenesis in rats and induced teratogenic effects in rats and in rabbits.

**Appears This Way
On Original**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 50-808

Review number: 1

Sequence number/letter date/type of submission: N-000/30-JUN-2005

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Medicis Pharmaceutical Corp.

Manufacturer for drug substance: _____

Reviewer name: Norman A. See, Ph.D.

Division name: Division of Dermatology and Dental Products

HFD #: 540

Review completion date: 22-FEB-2006

Drug:

Trade name: Solodyn _____

Generic name: Minocycline HCl, USP

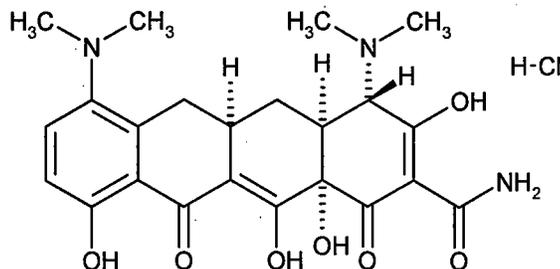
Code name: NA

Chemical name: 4,7-bis-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenicarboxamide hydrochloride

CAS registry number: 13614-98-7

Molecular formula/molecular weight: $C_{23}H_{27}N_3O_7 \cdot HCl$ _____

Structure:



Relevant INDs/NDAs/DMFs: IND 65,398. Minocycline has been associated with previously approved NDAs, but this application does not reference those NDAs.

Drug class: Antibiotic

Intended clinical population: Patients with acne vulgaris

Clinical formulation (sustained-release): Three formulations of extended-release (consisting of minocycline core units with an opadry film coating) have been proposed for marketing:

Extended-release formulations (units are mg per):

| Component | 45 mg Formulation | 90 mg Formulation | 135 mg Formulation |
|----------------------------------|-------------------|-------------------|--------------------|
| Minocycline HCl, USP | 45.0 | 90.0 | 135.0 |
| Lactose monohydrate, NF | | | |
| Hypromellose, Type 2910, USP | | | |
| Colloidal silicon dioxide, NF | | | |
| Magnesium stearate, NF | | | |
| Opadry II Film Coat ¹ | | | |
| Total | 412.0 | 412.0 | 412.0 |

¹ Opadry II gray, Opadry II yellow, and Opadry II pink, used for the 45 mg, 90 mg, and 135 mg formulations, respectively.

Route of administration: Oral.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: Note: This product was developed under IND 65,398, which was originally submitted 12-JUL-2002. Some of the studies which support this NDA were reviewed under review formats that were in use at the time the data were originally submitted. Reviews of those studies are included in this NDA in the format under which those studies were originally reviewed and signed off.

Safety Pharmacology:

1. Minocycline Irwin study in rats including body temperature and locomotor assessment (oral administration), study No. VTK-002/033607.
2. Minocycline telemetric evaluation of cardiovascular effects in the conscious dog, study No. VTK-007/042650.
3. Minocycline evaluation of respiratory parameters in the conscious rat using whole body bias flow plethysmography (oral administration), study No. VTK-008/042327.

Repeat-Dose Toxicology:

1. Minocycline hydrochloride preliminary toxicity study by oral gavage administration to CD-1 mice for 13 weeks, study No. VTK-006/042123.
2. Minocycline hydrochloride preliminary toxicity study by oral gavage administration to CD rats for 13 weeks, study No. VTK-005/042334.
3. Minocycline hydrochloride toxicity study by oral gavage administration to Cynomolgus monkeys for up to 13 weeks, study No. VTK-009/043216.
4. A 9-month oral (nasogastrically) study of minocycline hydrochloride in Cynomolgus monkeys, study No. ~~—~~-450012 (unaudited preliminary report submitted).

Genetic Toxicology:

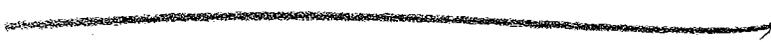
1. Bacterial reverse mutation assay, study No. AA57RE.503.BTL.
2. In vitro mammalian chromosome aberration test, study No. AA57RE.341.BTL.
3. In vitro mammalian cell gene mutation test (CHO/HGPRT assay), study No. AA57RE.782.BLT.
4. Mammalian erythrocyte micronucleus test, study No. AA57RE.123.BTL.

Carcinogenicity:

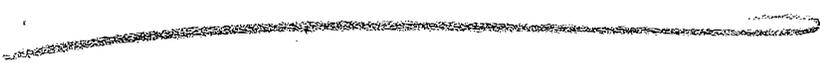
To be evaluated post-approval.

Reproductive Toxicology:

1. A study of fertility and early embryonic development to implantation of minocycline hydrochloride in rats, study No. ~~—~~-450005.
 2. A study of the effects of minocycline hydrochloride on embryo/fetal development in rats, study No. ~~—~~-450009.
 3. A study of the effects of minocycline hydrochloride on embryo/fetal development in rabbits, study No. ~~—~~-450011.
 4. A study of the effects of minocycline hydrochloride on pre- and postnatal development, including maternal function in rats, study No. ~~—~~-450007.
-



2
3



2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Minocycline hydrochloride inhibits the growth of certain species of bacteria through inhibition of protein synthesis. Reduction of the bacterial count may reduce the size and quantity of lesions by reducing inflammation.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Minocycline inhibits the growth of certain species of bacteria through inhibition of protein synthesis by blocking aminoacyl-t-RNA binding to the m-RNA-ribosome complex. Minocycline is active against a number of gram-positive and gram-negative organisms, including *Propionibacterium acnes*. The mechanism through which minocycline ameliorates acne is not fully elucidated, although reducing the bacterial count may reduce the size and quantity of lesions by reducing inflammation.

Drug activity related to proposed indication: Minocycline presumably ameliorates acne by inhibiting the growth of pathogenic bacteria, such as *Propionibacterium acnes*, which prevents those organisms from inducing inflammatory lesions.

2.6.2.3 Secondary pharmacodynamics

None.

2.6.2.4 Safety pharmacology

Neurological effects: Minocycline was evaluated for effects on behavior and psychological state in rats in an Irwin test. Minocycline was orally administered once to fasted rats at exposures of 0 (control), 10, 100, or 1000 mg/kg (4 male rats at each exposure level, 10 mL/kg dose volume). The parameters defined in the Irwin test (e.g., movement, apathy, fighting, tremor, convulsions, respiration, alertness, startle response, righting reflex, gait, corneal reflex, passivity, aggressiveness, salivation, lacrimation, pain response, grooming, etc.) were evaluated at 30, 90, 150, and 300 minutes post-dosing and again the next day. No clearly treatment-related changes in behavior were observed in any group, although one high-dose rat displayed slight tremor at 90 minutes post-dosing, and some high-dose animals may have had slightly reduced locomotor activity. It was concluded that minocycline at dosages up to 1000 mg/kg produced no significant changes in behavior or psychological state in rats.

Cardiovascular effects: Minocycline was orally administered to fasted, conscious beagle dogs (2/sex) at exposures of 0 (control), 10, 50, or 100 mg/kg. The animals had previously had telemetry transducers surgically implanted to monitor arterial blood pressure and "lead II" ECG variables (RR, PR, and QT intervals and QRS duration). Minocycline caused no effects on gross behavior, arterial blood pressure, heart rate, or the ECG, although an exposure of 100 mg/kg induced diarrhea, vomiting, and hunched posture in 3 of 4 animals.

Pulmonary effects: Minocycline was orally administered once to fasted rats at exposures of 0 (control), 10, 100, or 1000 mg/kg (8 male rats at each exposure level, 10 mL/kg dose volume). Morphine, 200 mg/kg PO, was administered to an additional group as a positive control material. The animals were placed in whole-body plethysmography chambers and respiratory rate, tidal volume, and minute volume were measured at 30, 90, 150, and 300 minutes post-dosing. No effects on respiration were observed at 10 or 100 mg/kg. An exposure of 1000 mg/kg significantly reduced respiratory rate and minute volume at 30 minutes post-dosing, and significantly increased tidal volume at 30, 90, and 150 minutes post-dosing. Morphine reduced the respiratory rate, as expected.

Renal effects: Not evaluated.

Gastrointestinal effects: Not evaluated.

Abuse liability: Not evaluated.

Other: NA

Safety pharmacology summary: Minocycline was evaluated for effects on behavior and psychological state in rats in an Irwin test, for cardiovascular effects in conscious dogs, and for effects on respiration in rats. Minocycline had no apparent effects on behavior or psychological state, or on arterial blood pressure, heart rate, or the ECG, but (at a clinically irrelevant level of exposure of 1000 mg/kg) significantly reduced respiratory rate and minute volume and significantly increased tidal volume.

Safety pharmacology conclusions: Minocycline had no effect on behavior, psychological state, arterial blood pressure, heart rate, the ECG, or (at clinically relevant levels of exposure) respiration.

Neurological effects: None known.

Cardiovascular effects: None known.

Pulmonary effects: None known.

Renal effects: None known.

Gastrointestinal effects: None known.

Abuse liability: None known.

Other: None

2.6.2.5 Pharmacodynamic drug interactions

None known.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not available.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Minocycline is well absorbed from the GI tract, with peak serum levels observed within two to four hours. Greater than 95% of an oral dose is typically absorbed. Minocycline is highly lipid soluble, and exhibits a large volume of distribution (>80 L in humans). Minocycline is approximately 70% to 80% bound to plasma proteins, exhibits a serum half-life of 11 to 18 hours, and a renal clearance rate of 9 mL/min. Minocycline is

metabolized in the liver to three inactive metabolites (9-hydroxy-, N-desmethyl-, and N4-desmethyl-minocycline). Minocycline and its metabolites are primarily eliminated in the feces.

2.6.4.2 Methods of Analysis

Minocycline concentrations in nonclinical samples were analyzed using HPLC and UV detection techniques. The methods used were adequately validated and had acceptable limits of quantitation.

2.6.4.3 Absorption

Ingested minocycline is rapidly and nearly completely absorbed from the GI tract, with peak serum levels observed within two to four hours. Greater than 95% bioavailability is observed. Studies were conducted in mice, rats, and monkeys in which the animals were orally dosed and toxicokinetic data were obtained. The NDA also contains pharmacokinetic data from the literature concerning dogs that were exposed to minocycline. Systemic exposure data obtained from pivotal safety studies involving mice, rats, and monkeys, in comparison to clinical exposure, is summarized in the table presented in section 2.6.7 of this review.

2.6.4.4 Distribution

Minocycline is distributed throughout the body, and tends to accumulate in fat. Following IV administration of ¹⁴C-minocycline to dogs, activity was highest in the ileum, colon, and gall bladder, followed by liver, thyroid, kidney, lungs, and pancreas. Minocycline is approximately 70% to 80% bound to plasma proteins.

2.6.4.5 Metabolism

In humans, minocycline is metabolized in the liver to three inactive metabolites (9-hydroxy-, N-desmethyl-, and N4-desmethyl-minocycline). Rats excrete minocycline primarily as the parent compound and as N-desmethyl-minocycline. Dogs apparently do not extensively metabolize minocycline.

2.6.4.6 Excretion

Minocycline (and metabolites thereof) are primarily excreted in the feces, with a small percentage being excreted in urine. In rats greater than 90% of an intraperitoneal dose was excreted in the feces, while in dogs approximately 80% to 90% of a parenteral dose is excreted in the feces.

2.6.4.7 Pharmacokinetic drug interactions

None known.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable.

2.6.4.9 Discussion and Conclusions

Minocycline is well absorbed following oral administration. Peak serum levels are observed within two to four hours. Minocycline is highly lipid soluble and distributes throughout the body. Minocycline is approximately 70% to 80% bound to plasma proteins and exhibits a serum half-life of 11 to 18 hours. Minocycline is metabolized in the liver; metabolism of the compound varies between species. Minocycline and its metabolites are primarily eliminated in the feces.

2.6.4.10 Tables and figures to include comparative TK summary

Interspecies Comparison of Pharmacokinetic Parameters at Steady State

| Species | Dose (mg/kg/day) | T _{max} of ♂ (hr) | T _{max} of ♀ (hr) | C _{max} of Males (ng/mL) | C _{max} of Females (ng/mL) | AUC of Males (ng·hr/mL) | AUC of Females (ng·hr/mL) |
|---------|------------------|----------------------------|----------------------------|-----------------------------------|-------------------------------------|-------------------------|---------------------------|
| Mouse | 50 | 1 | 1 | 4823 | 5263 | 23577 | 23829 |
| Mouse | 200 | 4 | 8 | 18600 | 21803 | 101482 | 169356 |
| Mouse | 500 | 8 | 1 | 22867 | 57390 | 205244 | 217260 |
| Rat | 5 | 4 | 2 | 2930 | 2820 | 22260 | 19970 |
| Rat | 15 | 1 | 2 | 11670 | 11700 | 64230 | 84340 |
| Rat | 50 | 4 | 4 | 34770 | 37530 | 245360 | 245340 |
| Monkey | 10 | 2 | 2 | 6640 | 7000 | 90070 | 91420 |
| Monkey | 50 | 2 | 4 | 11740 | 25600 | 160370 | 341210 |
| Monkey | 130 | 4 | 4 | 46800 | 43530 | 644420 | 712150 |
| Human | 1* | 3 | 3 | 2630** | 2630** | 33,320** | 33,320** |

*Human data are from studies conducted with the to-be-marketed formulation of Solodyn 135 mg ~~in~~ in healthy adults (7 men, 21 women). The recommended dosage is approximately 1 mg/kg/day, although the actual exposure in this clinical study was probably closer to 1.5 to 2 mg/kg/day.

**The clinical data are average values for males and females combined.

See section 2.6.7 of this review for a tabulated comparison of systemic exposure levels at the NOAEL in selected studies.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See above.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: In mice that were treated for 13 weeks at exposures up to 500 mg/kg/day, treatment-induced toxicity included reduced survival, reduced body weight gain (significant in females only), significantly elevated hematocrit and hemoglobin, increased mean thyroid weight, dark appearance of the thyroids, fine, granular dark-brown pigmentation in the cytoplasm of follicular cells, and follicular cell hypertrophy in the thyroid. All of these effects were observed in both the mid-dose and high-dose groups (200 and 500 mg/kg/day, respectively), but the only abnormal observation in low-dose animals (50 mg/kg/day) was the presence of pigment and staining of the thyroid. Plasma levels of T4 and TSH were significantly elevated in HD males only.

Little toxicity was observed in rats that were treated for 13 weeks at exposures ranging from 5 to 50 mg/kg/day. As in mice, treatment-related effects at an exposure of 50 mg/kg/day were apparently limited to deposition of pigment in the thyroid (in all treatment groups), increased mean weight of the thyroid, and discoloration of the thyroid, teeth, and bones. However, this pigment deposition/discoloration did not appear to correlate with toxicologically meaningful changes in form or function.

Similarly, little toxicity was observed in Cynomolgus monkeys treated for 13 weeks at exposures up to 130 mg/kg/day. Treatment-related effects were apparent in the thyroid, liver, and small intestine, including altered levels of hormones related to the thyroid (elevated TSH, reduced T3), pigment deposition within the thyroid, follicular cell hypertrophy of the thyroid, minimal centrilobular hepatocyte enlargement, and minimal to slight vacuolation of the lamina propria and lacteals of the small intestine. In the absence of effects on body weight or survival, these effects do not appear to have been life-threatening. Although toxicity observed in all groups was not severe, I consider the lowest exposure studied, 10 mg/kg/day, to have been a NOAEL, since minimal centrilobular hepatocyte enlargement was observed with increased frequency and in both genders at higher levels. Dark pigment deposition occurred in the thyroid in all groups.

The primary toxicological target organ for minocycline in all species appears to be the thyroid, with treatment-related effects on the thyroid manifesting (at sufficient levels of exposure) as grossly visible enlargement, increased mean weight, dark red discoloration, increased colloid content, accumulation of brown pigment in the follicular cells, follicular cell hypertrophy, and elevated plasma levels of T4 and TSH. These effects are of a minimal to mild severity in most instances, and do not appear to be toxicologically relevant at levels of exposure close to those observed clinically.

Genetic toxicology: Minocycline was negative in three in vitro genetic toxicology assays, including an Ames assay, an assay for chromosomal aberrations in human peripheral blood lymphocytes, and a mutation (HGPRT) assay in cultured CHO cells, and in an in vivo assay for chromosomal aberrations (micronucleus assay). Minocycline is apparently not genotoxic.

Carcinogenicity: To be evaluated post-approval.

Reproductive toxicology: Male and female reproductive performances were unimpaired in a study in which F0 male rats were dosed for 28 days prior to pairing and continuing throughout the remainder of the study and F0 females were dosed from 14 days prior to pairing and continuing until day 7 of gestation. There were no effects on the percentages of animals that copulated or became pregnant, or on latency to mating. Spermatogenic endpoints were adversely affected at an exposure of 300 mg/kg/day, including a significant reduction in the mean number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and increased numbers of morphologically abnormal sperm cells. The latter effect was also observed at an exposure of 100 mg/kg/day. Morphological abnormalities observed in sperm samples included absent heads, misshapen heads, and abnormal flagella. No differences in intrauterine survival were observed, including no effects on pre- or post-implantation losses or numbers of viable embryos. An exposure of 30 mg/kg/day was considered to be the NOAEL for male reproductive toxicology under the conditions of this study, due to effects on sperm morphology at 100 mg/kg/day. An exposure of 300 mg/kg/day was considered to be the NOAEL for female reproductive toxicology under the conditions of this study.

Minocycline was assessed for teratogenic effects. When administered to female rats on days 6-17 of gestation, minocycline reduced the mean fetal weight and induced skeletal malformations (bent limb bones) and skeletal variations (reduced skeletal ossification). Maternal body weight gain was significantly reduced during the period of dosing. The NOAEL for maternal toxicity in rats was 10 mg/kg/day, due to effects on mean weight gain at 30 mg/kg/day and above. An exposure of 10 mg/kg/day (the lowest exposure evaluated for teratogenic effects in rats) was essentially a NOAEL for fetal toxicity, although slightly (but statistically significantly) reduced fetal body weight was observed in the LD group. When administered to female rabbits on days 7-20 of gestation at exposures up to 175 mg/kg/day, minocycline induced abortion in a minority of does, reduced maternal weight gain, gravid uterine weight, and mean fetal body weight, and induced skeletal malformations (bent limb bones). The NOAEL in rabbits for maternal and fetal toxicity was 50 mg/kg/day.

Minocycline was assessed for effects on pre- and postnatal development, including maternal function in rats. Minocycline was administered to pregnant female rats from day 6 of gestation through the period of lactation (postpartum day 20). Body weight gain was significantly reduced in high-dose F0 females during the period of treatment. There was no effect of treatment on the duration of the gestation period or on the number of dams with all pups dying postpartum. There was no effect on the mean number of pups born or the number of viable pups per litter. Postnatal survival was decreased with increasing exposure to minocycline. Gross external anomalies observed in F1 pups from the HD group (50 mg/kg/day; observed at higher incidence than in the control group) included smallness of size, malrotated forelimbs, and micromelia. No effects were observed on the physical development (time to balanopreputial separation or vaginal

opening), behavior, learning ability, or reproduction of F1 pups, and there was no effect on gross appearance of F2 pups.

Special toxicology: Not applicable.

2.6.6.2 Single-dose toxicity

No single-dose toxicology studies were submitted.

2.6.6.3 Repeat-dose toxicity

2.6.6.3.1 Study title: Minocycline hydrochloride preliminary toxicity study by oral gavage administration to CD-1 mice for 13 weeks.

Key study findings: Treatment-induced toxicity observed in this study included reduced survival, reduced body weight gain (significant in females only), significantly elevated hematocrit and hemoglobin, increased mean thyroid weight, dark appearance of the thyroid, fine, granular dark-brown pigmentation in the cytoplasm of follicular cells, and follicular cell hypertrophy in the thyroid. All of these effects were observed in both the mid-dose and high-dose groups (200 and 500 mg/kg/day, respectively), but the only abnormal observation in low-dose animals (50 mg/kg/day) was the presence of pigment and staining of the thyroid. Plasma levels of T4 and TSH were significantly elevated in HD males only. Toxicokinetic data confirmed that systemic exposure to the test article increased roughly in proportion to the oral dose. Toxicokinetic parameters in males and females were similar.

Study No.: VTK-006/042123

Document #, Volume #, and Page #: NA

Conducting laboratory and location: _____

Date of study initiation: 23-JAN-2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline hydrochloride, Lot No. 329.55-

Formulation/vehicle: Minocycline HCl was dissolved in water.

Methods (unique aspects):

Dosing:

Study overview:

| Group/Treatment | Exposure Level* (mg/kg/day) | Number/sex in Main Study | Number/sex in Toxicokinetic Group |
|-----------------|-----------------------------|--------------------------|-----------------------------------|
| | | | |

| | | | |
|--------------|-----|----|----|
| 1. Control | 0 | 12 | 0 |
| 2. Low Dose | 50 | 12 | 54 |
| 3. Mid Dose | 200 | 12 | 54 |
| 4. High Dose | 500 | 12 | 54 |

*Exposures are expressed in terms of minocycline base. The exposures to the hydrochloride salt were 0, 57.8, 231.2, and 578.0 mg/kg/day, respectively.

Species/strain: Mouse CD-1 (ICR) BR

#/sex/group or time point (main study): 12/sex/group, +54/sex/group used for TK analysis (groups 2-4 only)

Satellite groups used for toxicokinetics or recovery: yes, see above

Age: Six weeks at start of treatment

Weight: At start of dosing: males, approx. 30 g; females, approx. 26 g

Doses in administered units: 0 (vehicle control); 50 mg/kg/day; 200 mg/kg/day; and 500 mg/kg/day.

Route, form, volume, and infusion rate: Oral (gavage), 10 ml/kg, once per day for approximately 92 consecutive days.

Observations and times:

Clinical signs: Main study and recovery animals observed twice daily for general health and at least once weekly for clinical signs of toxicity

Body weights: Weekly

Food consumption: Weekly

Ophthalmology: No

EKG: No

Hematology: Assessed in all main-study animals during week 13 of treatment.

Clinical chemistry: Assessed in all main-study animals at termination.

Urinalysis: No

Gross pathology: All main-study animals.

Organs weighed: Adrenals, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, uterus with cervix.

Histopathology: A full range of tissues (including gross lesions) from main-study animals in the control and HD groups was examined, plus the thyroids/parathyroids from the LD and MD groups, plus a full range of tissues from all animals that died on-study.

Toxicokinetics: Blood samples were collected from three TK animals per gender per group for toxicokinetic analysis pre-dose and 1, 2, 4, 8, 12, 16, 20, and 24 hours post-dosing on day 1 and during week 13.

Other: Plasma thyroid hormone (T3, T4, and TSH) levels assessed in 6 animals/sex/group at termination. Sperm samples were obtained from the vas deferens of all main-study males at termination and were analyzed for motility and morphology. The cauda epididymis and left testis of each male were homogenized and assessed for the concentration of sperm and spermatids, respectively.

Results:

- Mortality: Treatment-related deaths were observed in the MD and HD groups only. Combining data from the main-study and toxicokinetic groups, the unscheduled deaths included two MD males (in weeks 1 and 11), two MD females (in weeks 1 and 2), six HD males (in weeks 1, 7 (2 animals died), 10, 11, and 12) and one HD female (in week 2).
- Clinical signs: Abnormal signs in animals that survived to scheduled sacrifice were transient and of minor severity, but included distended abdomen, rales, skin pallor, and piloerection. Signs observed in early decedents exhibited swollen and firm abdomen, partially closed eyes, hunched posture, slow/deep respiration, thin build, and hypoactivity.
- Body weight/weight gain: Slightly reduced in proportion to exposure; weight gain significantly reduced in MD and HD females:

Mean body weights of unfasted animals, week 13 (grams; mean±SD):

| Group | Males | Females |
|-----------------|-----------|-----------|
| Vehicle control | 40.4±3.12 | 32.5±3.34 |
| 50 mg/kg/day | 39.0±3.18 | 30.9±2.53 |
| 200 mg/kg/day | 39.4±4.20 | 30.5±4.29 |
| 500 mg/kg/day | 39.2±2.70 | 29.8±2.28 |

Approximate mean body weight gains over weeks 0-13 (grams):

| Group | Males | Females |
|-----------------|----------|------------|
| Vehicle control | 8.6±2.11 | 8.0±3.19 |
| 50 mg/kg/day | 8.4±2.41 | 6.2±1.79 |
| 200 mg/kg/day | 7.7±3.35 | 5.0±3.56* |
| 500 mg/kg/day | 7.7±1.82 | 4.1±1.78** |

*p<0.05; **p<0.01

- Food consumption: No remarkable observations.
- Ophthalmology: NA
- EKG: NA
- Hematology: Hematocrit and hemoglobin values were slightly but significantly elevated in MD and HD males and in HD females. The values were close to the normal range, and it is doubtful that these observations were toxicologically meaningful. No other remarkable observations.
- Clinical chemistry: No remarkable observations. A few statistically significant differences between high-dose and control animals were observed, but the aberrant values were within the range of historical control values and the differences were generally small.
- Urinalysis: NA

- Organ Weights: The mean absolute weight of the thyroid increased in proportion to exposure in both genders:

Mean absolute weight (g±SD):

| Gender/Dose Group (mg/kg/day) | Thyroid |
|-------------------------------|-----------------|
| Males/Control | 0.0051±0.0008 |
| Males/50 | 0.0059±0.0011 |
| Males/200 | 0.0064±0.0012** |
| Males/500 | 0.0087±0.0012** |
| Females/Control | 0.0052±0.0006 |
| Females/50 | 0.0055±0.0011 |
| Females/200 | 0.0064±0.0011** |
| Females/500 | 0.0088±0.0018** |

**p<0.01

Similar increases were observed after normalizing the data according to mean body weight or brain weight. No other remarkable observations.

- Gross pathology: Dark appearance of the thyroids was observed in all treatment groups (but not in controls); the in-group incidence increased in proportion to exposure. Yellow discoloration of bones and teeth was noted in all treatment groups, but not in controls. No other remarkable observations.
- Histopathology: Among animals that survived to scheduled sacrifice, the only treatment-related observations occurred in the thyroid, and included the presence of fine, granular dark-brown pigmentation in the cytoplasm of follicular cells, pigmented cells in the lumen, pigmented macrophages, and follicular cell hypertrophy. The incidence and severity of these changes increased with increasing exposure to minocycline. Additional findings that were only apparent among animals found dead or sacrificed in extremis included inflammation and hemorrhage of the stomach and involution of the thymus.
- Toxicokinetics:

Toxicokinetic Parameters Calculated from Data Obtained During Study Week 13

| Gender/Dose Group (mg/kg/day) | AUC _{inf} (ng•hr/mL) | C _{max} (ng/mL) | T _{1/2} (hr) | T _{max} (hr) |
|-------------------------------|-------------------------------|--------------------------|-----------------------|-----------------------|
| Males/50 | 23577 | 4823 | 3.91 | 1 |
| Males/200 | 101482 | 18600 | 5.39 | 4 |
| Males/500 | 205244 | 22867 | 3.05 | 8 |
| Females/50 | 23829 | 5263 | 2.67 | 1 |
| Females/200 | 169356 | 21803 | 2.82 | 8 |
| Females/500 | 217260 | 57390 | 2.90 | 1 |

Group sizes of 3 animals per time point. Standard deviations not available.

- Other: Plasma levels of T4 and TSH were significantly elevated in HD males (T4 was 62 ± 5.6 nmol/L in controls and 112 ± 21.6 nmol/L in HD males, $p, 0.01$; TSH was 5.9 ± 0.44 ng/mL in controls and 7.2 ± 1.20 ng/mL in HD males, $p, 0.01$). No remarkable observations on sperm motility, morphology, or number.

2.6.6.3.2 Study Title: Minocycline hydrochloride preliminary toxicity study by oral gavage administration to CD rats for 13 weeks

Key study findings: Little toxicity was observed under the conditions of this study. Treatment-related effects were apparently limited to deposition of pigment in the thyroid (in all treatment groups), increased mean weight of the thyroid, and discoloration of the thyroid, teeth, and bones. However, this pigment deposition/discoloration did not appear to correlate with toxicologically meaningful changes in form or function. Toxicokinetic data confirmed that systemic exposure to the test article increased roughly in proportion to the oral dose. Toxicokinetic parameters in males and females were similar. Although pigmentation of the thyroid was observed at all treatment levels (and increased in incidence and severity with increasing exposure to minocycline), this effect does not appear to be dose-limiting, and the high-dose examined in this study (50 mg/kg/day) could be regarded as being a no adverse effect level (NOAEL).

Study No.: VTK-005/042334

Document #, Volume #, and Page #: NA

Conducting laboratory and location: _____

Date of study initiation: 10-FEB-2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline hydrochloride, Lot No. 329.55 —

Formulation/vehicle: Minocycline HCl was dissolved in water.

Methods (unique aspects):

Dosing:

Study overview:

| Group/Treatment | Exposure Level* (mg/kg/day) | Number/sex in Main Study | Number/sex in Toxicokinetic Group |
|-----------------|-----------------------------|--------------------------|-----------------------------------|
| 1. Control | 0 | 10 | 0 |
| 2. Low Dose | 5 | 10 | 9 |
| 3. Mid Dose | 15 | 10 | 9 |
| 4. High Dose | 50 | 10 | 9 |

*Exposures are expressed in terms of minocycline base. The exposures to the hydrochloride salt were 0, 5.78, 17.34, and 57.80 mg/kg/day, respectively.

Species/strain: Rat— :CD (SD)IGS BR

#/sex/group or time point (main study): 10/sex/group, +9/sex/group used for TK analysis (groups 2-4 only)

Satellite groups used for toxicokinetics or recovery: yes, see above

Age: Seven weeks at start of treatment

Weight: At start of dosing: males, approx. 250 g; females, approx. 200 g

Doses in administered units: 0 (vehicle control); 5 mg/kg/day; 15 mg/kg/day; and 50 mg/kg/day.

Route, form, volume, and infusion rate: Oral (gavage), 10 ml/kg, once per day for approximately 91 consecutive days.

Observations and times:

Clinical signs: Main study animals observed twice daily for general health and at least once weekly for clinical signs of toxicity

Body weights: Weekly

Food consumption: Weekly

Ophthalmology: No

EKG: No

Hematology: Assessed in 5 main-study animals per group (those not used for assessment of thyroid parameters) during week 13 of treatment following overnight fasting.

Clinical chemistry: Assessed in 5 main-study animals per group (those not used for assessment of thyroid parameters) during week 13 of treatment following overnight fasting.

Urinalysis: No

Gross pathology: All main-study animals.

Organs weighed: Adrenals, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, uterus with cervix.

Histopathology: A full range of tissues (including gross lesions) from main-study animals in the control and HD groups was examined, plus the testes, thyroids/parathyroids, and liver (males only) from the LD and MD groups.

Toxicokinetics: Blood samples were collected from nine TK animals per gender per group for toxicokinetic analysis pre-dose and 1, 2, 4, 8, 12, 16, 20, and 24 hours post-dosing on day 1 and during week 13.

Other: Plasma thyroid hormone (T3, T4, and TSH) levels assessed in 5 animals/sex/group at baseline and during week 13. Sperm samples were obtained from the vas deferens of all main-study males at termination and were analyzed for motility and morphology. The cauda epididymis and left testis of each male were homogenized and assessed for the concentration of sperm and spermatids, respectively.

Results:

- Mortality: All animals survived to scheduled sacrifice.
- Clinical signs: Dark teeth were observed in HD animals and in MD females beginning approximately day 45.
- Body weight/weight gain: No statistically significant differences. Body weight gain trended toward reduction in LD and HD males (approaching 10%), but in the absence of dose-dependency (reduction in LD but not MD males) or effects in females it must be concluded there was no genuine effect:

Mean body weights of unfasted animals, week 13 (grams; mean±SD):

| Group | Males | Females |
|-----------------|------------|------------|
| Vehicle control | 598.4±51.1 | 315.0±25.8 |
| 5 mg/kg/day | 560.9±61.8 | 315.0±20.2 |
| 15 mg/kg/day | 587.7±61.1 | 312.3±27.8 |
| 50 mg/kg/day | 568.8±35.0 | 320.3±26.6 |

None significant.

Approximate mean body weight gains over weeks 0-13 (grams):

| Group | Males | Females |
|-----------------|------------|------------|
| Vehicle control | 335.7±40.7 | 120.6±21.6 |
| 5 mg/kg/day | 302.4±45.8 | 121.8±14.0 |
| 15 mg/kg/day | 326.7±49.3 | 122.5±23.8 |
| 50 mg/kg/day | 305.7±32.5 | 126.2±21.4 |

None significant.

- Food consumption: No remarkable observations.
- Ophthalmology: NA
- EKG: NA
- Hematology: No remarkable observations.
- Clinical chemistry: No remarkable observations.
- Urinalysis: NA
- Organ Weights: The mean absolute weight of the thyroid increased in HD, but not LD or MD, animals of both genders:

Mean absolute weight (g±SD):

| Gender/Dose Group (mg/kg/day) | Thyroid |
|-------------------------------|---------------|
| Males/Control | 0.025±0.005 |
| Males/5 | 0.025±0.005 |
| Males/15 | 0.027±0.005 |
| Males/50 | 0.032±0.004** |
| Females/Control | 0.0196±0.0016 |

| | |
|------------|-----------------|
| Females/5 | 0.0192±0.0027 |
| Females/15 | 0.0220±0.0024 |
| Females/50 | 0.0235±0.0032** |

**p<0.01

Similar increases were observed after normalizing the data according to mean body weight or brain weight. A few minor differences in mean organ weights were observed, although these were generally within historical control ranges. In males, these included trends toward reduced mean absolute weights of the epididymides (which reached statistical significance for the HD group), spleen (statistically significant for MD and HD males), and thymus (statistically significant for HD males). In females, these included slightly increased adrenal weight (statistically significant for HD females) and increased liver weight (statistically significant for LD, MD and HD females). These differences were small and, in the absence of corresponding clinical pathological or histopathological observations, probably not toxicologically relevant.

- Gross pathology: Dark appearance of the thyroids was observed in essentially all treated animals, but not in controls. Yellow discoloration of bones was noted in all treatment groups, but not in controls. Yellow discoloration of teeth was noted in the MD and HD groups, but not in controls. No other remarkable observations.

- Histopathology: In the thyroid, fine, dark-brown pigment granules were observed in the cytoplasm of follicular cells from rats of both genders from all treatment groups, but not in controls. The severity (quantity of pigment) increased with increasing exposure to minocycline. Pigmented cells were observed in the lumen of the follicles in MD and HD animals of both genders and in LD males. Follicular cell hypertrophy was observed in animals of all groups; the incidence and severity increased with increasing exposure to minocycline.

Vacuolation of hepatocytes was observed in four HD male rats only.

- Toxicokinetics:

Toxicokinetic Parameters Calculated from Data Obtained During Study Week 13

| Gender/Dose Group (mg/kg/day) | AUC _{inf} (ng•hr/mL) | C _{max} (ng/mL) | T _{1/2} (hr) | T _{max} (hr) |
|-------------------------------|-------------------------------|--------------------------|-----------------------|-----------------------|
| Males/5 | 22260 | 2930 | 3.91 | 4 |
| Males/15 | 64230 | 11670 | 4.44 | 1 |
| Males/50 | 245360 | 34770 | 5.33 | 4 |
| Females/5 | 19970 | 2820 | 4.25 | 2 |
| Females/15 | 84340 | 11700 | 5.37 | 2 |
| Females/50 | 245340 | 37530 | 4.24 | 4 |

Group sizes of 3 animals per time point. Standard deviations not available.

- Other: Plasma levels of T3, T4 and TSH did not differ between groups.

No remarkable observations on sperm motility, morphology, or number.

2.6.6.3.3 Study title: Minocycline hydrochloride toxicity study by oral gavage administration to Cynomolgus monkeys for up to 13 weeks

Key study findings: Little toxicity was observed under the conditions of this study. Treatment-related effects were apparent in the thyroid, liver, and small intestine, including altered levels of hormones related to the thyroid (elevated TSH, reduced T3), pigment deposition within the thyroid, follicular cell hypertrophy of the thyroid, minimal centrilobular hepatocyte enlargement, and minimal to slight vacuolation of the lamina propria and lacteals of the small intestine. Toxicokinetic data confirmed that systemic exposure to the test article increased roughly in proportion to the oral dose. Toxicokinetic parameters in males and females were similar. In the absence of effects on body weight or survival, these effects do not appear to have been life-threatening. Although toxicity observed in all groups was not severe, I consider the lowest exposure studied, 10 mg/kg/day, to have been a NOAEL, since minimal centrilobular hepatocyte enlargement was observed with increased frequency and in both genders at higher levels. Dark pigment deposition within the thyroid occurred in all groups, but this does not seem to be associated with other pathology.

Study No.: VTK 009/043216

Document #, Volume #, and Page #: NA

Conducting laboratory and location: _____

Date of study initiation: 26-MAY-2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline hydrochloride, Lot No. 329.55

Formulation/vehicle: Minocycline HCl was dissolved in water.

Methods (unique aspects):

Dosing:

Study overview:

| Group/Treatment | Exposure Level ^{1,2} (mg/kg/day) | Number/sex in Study |
|-----------------|--|------------------------|
| 1. Control | 0 | 3 |
| 2. Low Dose | 10 | 3 |
| 3. Mid Dose | 30 (Days 1-14) 50 (Days 15-91) | 3 |
| 4. High Dose | 30 (Days 1-7) 50 (Days 8-14) 70 (Days 15-25) 100 (Days 26-46) | 3 |

| | | |
|--|------------------|--|
| | 130 (Days 47-91) | |
|--|------------------|--|

¹Exposures are expressed in terms of minocycline base. Multiply the quantity of base by 1.156 to calculate the quantity of the hydrochloride salt administered.

²The daily exposures for the mid and high-dose groups were increased gradually to avoid dose-limiting vomiting that had been observed in a preliminary study.

Species/strain: Cynomolgus monkey/NA

#/sex/group or time point (main study): 3/sex/group

Satellite groups used for toxicokinetics or recovery: No

Age: 15-16 months at start of treatment

Weight: At start of dosing: males, 2.34-2.84 kg; females, 1.79-2.34 kg

Doses in administered units: See table above.

Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg, once per day for approximately 91 consecutive days.

Observations and times:

Clinical signs: Animals observed twice daily for general health and at least once weekly for clinical signs of toxicity

Body weights: Daily

Food consumption: NA

Ophthalmology: Yes, all animals at baseline, groups 1 and 4 during week 13

EKG and blood pressure: Yes, all animals at baseline, groups 1 and 4 during week 13

Hematology: Yes, all animals, at baseline and during weeks 6 and 13

Clinical chemistry: Yes, all animals, at baseline and during weeks 6 and 13

Urinalysis: Yes, all animals, at baseline and during weeks 6 and 13

Gross pathology: All main-study animals

Organs weighed: Adrenals, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary, prostate, submandibular salivary glands, spleen, testes, thymus, thyroid/parathyroid, uterus with cervix.

Histopathology: A full range of tissues (including gross lesions) from animals in the control and HD groups was examined, plus the thyroids/parathyroids from the LD and MD groups.

Toxicokinetics: Blood samples were collected from all treated animals (not controls) for toxicokinetic analysis pre-dose and 1, 2, 4, 8, 12, 16, 20, and 24 hours post-dosing on day 1 and day 90.

Other: The plasma concentration of T3, T4, and TSH were assessed in all animals at baseline and during week 13.

Results:

- Mortality: All animals survived to scheduled sacrifice.
- Clinical signs: Post-dose vomiting was noted sporadically in animals receiving 50 mg/kg/day or greater. The animals generally became tolerant and stopped vomiting after a few days at a given dosage.
- Body weight/weight gain: No remarkable observations:

Mean body weights of unfasted animals, day 91 (kg; mean±SD):

| Group | Males | Females |
|-----------------|------------|------------|
| Vehicle control | 2.74±0.255 | 2.33±0.265 |
| Low Dose | 2.94±0.451 | 2.41±0.178 |
| Mid Dose | 2.89±0.260 | 2.35±0.246 |
| High Dose | 2.85±0.419 | 2.22±0.226 |

None significant.

Approximate mean body weight gains over weeks 0-13 (kg):

| Group | Males | Females |
|-----------------|------------|------------|
| Vehicle control | 0.20±0.165 | 0.14±0.104 |
| Low Dose | 0.44±0.312 | 0.28±0.027 |
| Mid Dose | 0.41±0.179 | 0.32±0.046 |
| High Dose | 0.36±0.347 | 0.08±0.133 |

None significant.

- Food consumption: NA
- Ophthalmology: No remarkable observations.
- EKG and BP: No remarkable observations.
- Hematology: No remarkable observations.
- Clinical chemistry: Levels of alkaline phosphatase and alanine aminotransferase were slightly increased in group 4 animals (approximately 1.5-2 fold).
- Urinalysis: No remarkable observations.
- Organ Weights: The mean absolute weights of the thyroid in all treatment groups, and of the liver in males, tended to be slightly increased, but the differences were not significant. In view of the histopathological observations of the thyroid and liver, the effects on organ weight may be been genuine and biologically significant.
- Gross pathology: Dark appearance of the thyroids was observed in all treated animals, but not in controls. Yellow discoloration of bones was noted in all treatment groups, but not in controls. No other remarkable observations.
- Histopathology:

Thyroid: Fine, dark-brown pigment granules were observed in the cytoplasm of follicular cells from animals of both genders from all treatment groups, but not in controls. The severity (quantity of pigment) increased with increasing exposure to minocycline. Pigmented cells were observed in the lumen of the follicles in MD and HD animals of both genders. Follicular cell hypertrophy was observed in animals of all groups; the incidence and severity increased with increasing exposure to minocycline.

Liver: Minimal centrilobular hepatocyte enlargement was observed in animals of both genders in the MD and HD groups, as well as one female in the LD group.

Small intestine: Minimal to slight vacuolation of the lamina propria and lacteals were observed throughout the small intestine; the incidence and severity increased with increasing exposure to minocycline.

- Toxicokinetics:

Toxicokinetic Parameters Calculated from Data Obtained on Study Day 90

| Gender/Dose Group (mg/kg/day) | AUC _{inf} (ng•hr/mL) | C _{max} (ng/mL) | T _{1/2} (hr) | T _{max} (hr) |
|-------------------------------|-------------------------------|--------------------------|-----------------------|-----------------------|
| Males/10 | 90070 | 6640 | 8.17 | 2 |
| Males/50 | 160370 | 11740 | 8.02 | 2 |
| Males/130 | 644420 | 46800 | 10.16 | 4 |
| Females/10 | 91420 | 7000 | 8.47 | 2 |
| Females/50 | 341210 | 25600 | 8.06 | 4 |
| Females/130 | 712150 | 43530 | 6.95 | 4 |

Group sizes of 3 animals per time point. Standard deviations not available.

- Other: Circulating levels of TSH were increased in relation to dosage, while levels of T3 were suppressed in group 4 males and females. There was no effect on T4 levels.

2.6.6.3.4 Study title: A 9-month oral (nasogastrically) study of minocycline hydrochloride in Cynomolgus monkeys

Note: The Division agreed in a meeting on 28-MAY-2003 that this study could be completed post-approval of the NDA; per the minutes of that meeting, "The chronic toxicology study will not be considered to be a filing issue, provided the initial submission to the NDA contains a protocol for the chronic toxicology study, a current interim report or update concerning that study, appropriate dose-ranging data to support the selection of dosages used, and a clear commitment to submit data from that study within a specific time frame. The sponsor will be expected to have initiated a suitable study prior to submission of a NDA". The NDA contains a protocol for the study (summarized below) and an interim report (the study was in week 25 at the time of the initial submission to the NDA, and an unaudited draft report of the study events to that time was submitted). An updated interim draft report was submitted 15-FEB-2006, when the study was in week 47. Dosing of all animals was completed at the end of week 39. Two animals per gender in the control and HD groups in the "recovery" group were being maintained without treatment for 90 days prior to sacrifice.

Key study findings through week 47, based on interim draft report: No unscheduled deaths occurred in the main study group, or in the recovery group through week 47 (week 8 of the recovery period). "Facial darkening" was noted in a dose-dependent manner in all treatment groups; this effect resolved during the recovery period. No apparent effects on body weight, ophthalmology, ECG, hematology, coagulation, urinalysis, or levels of TSH, T₃, or T₄ were observed. A statistically significant increase in alanine aminotransferase activity was observed in serum of HD animals at termination (week 39),

but the difference was small and probably not toxicologically significant (a 43% increase over the control value). HD animals exhibited grossly enlarged thyroids, increased mean weight of the thyroids, and (histologically) minimally to mildly increased colloid content in some HD animals. Dark red discoloration of the thyroid was observed in all treatment groups, which correlated histologically with minimal to mild accumulation of light brown pigment in the follicular cells. Accumulation of intracytoplasmic pigment was associated with minimal to mild follicular cell hypertrophy. Dark brown discoloration of the skin and yellow discoloration of bone was observed grossly in HD animals at week 39. These findings are preliminary. Final interpretation of these data will be regarded as being a post-approval commitment, and the study will be reviewed upon submission.

Study No.: — -450012

Document #, Volume #, and Page #: NA

Conducting laboratory and location: _____

Date of study initiation: 07-FEB-2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline hydrochloride, Lot No. 329.55 —
—, purity unknown at this time.

Formulation/vehicle: Minocycline HCl was dissolved in water.

Methods (unique aspects):

Dosing:

Study overview:

| Group/Treatment | Exposure Level ¹ (mg/kg/day) | Number/sex in Main Study | Number/sex in Recovery Group ² |
|-----------------|--|--------------------------------|---|
| 1. Control | 0 | 4 | 2 |
| 2. Low Dose | 5 | 4 | 0 |
| 3. Mid Dose | 10 | 4 | 0 |
| 4. High Dose | 30 | 4 | 2 |

¹Exposures are expressed in terms of minocycline base. Multiply the quantity of base by 1.156 to calculate the quantity of the hydrochloride salt administered.

²The original protocol specified maintaining animals in the "recovery" group for 28 days following cessation of dosing prior to sacrifice, but the interim report submitted on 15-FEB-2006 indicated that the duration of the recovery period had been extended to 90 days.

Species/strain: Cynomolgus monkey/NA

#/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: Yes (see table above)

Age: Approx. 2-3 years at start of treatment

Weight: Approx. 1.7-3.7 kg at start of dosing
Doses in administered units: See table above
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg, once per day for approximately 270 consecutive days.

Observations and times:

Clinical signs: Animals observed twice daily for general health and at least once weekly for clinical signs of toxicity
Body weights: Weekly
Food consumption: No
Ophthalmology: Yes, all animals at baseline and at termination
EKG: Yes, all animals at baseline and at termination
Hematology: Yes, all animals at baseline and at termination
Clinical chemistry: Yes, all animals at baseline and at termination
Urinalysis: Yes, all animals at baseline and at termination
Gross pathology: All animals
Organs weighed: Adrenals, brain, heart, kidneys, liver, pituitary, spleen, testes, thymus, thyroid/parathyroid, uterus with cervix.
Histopathology: A full range of tissues (including gross lesions) from all main-study animals plus recovery animals that may die prior to scheduled sacrifice. Recovery animals killed at scheduled sacrifice will be histopathologically evaluated if "target" tissues are observed in main-study animals.
Toxicokinetics: Blood samples were collected from all treated animals for toxicokinetic analysis pre-dose and 0.5, 1, 2, 4, 8, 12 and 24 hours post-dosing on days 0, 91, 182, and 266.
Other: The plasma concentration of T3, T4, and TSH assessed in all animals at baseline and at termination.

Results: Data from this study will be submitted and evaluated post-approval of the NDA.

2.6.6.4 Genetic toxicology**2.6.6.4.1 Study title:** Bacterial reverse mutation assay

Study No: AA57RE.503.BTL

Study Type: Ames test

Amendment #, Volume # and Page #: NA

Conducting Laboratory: ~~XXXXXXXXXXXXXXXXXXXX~~

Date of Study Initiation/completion: 23-APR-2002/12-SEP-2002

GLP Compliance: Yes

QA- Reports: Yes (X) No ()

Drug Lot Number: 06853

Study Endpoint: Growth in medium containing no histidine, indicating mutation from histidine dependence to histidine independence

Methodology:

- Strains/Species/Cell line: Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and E. coli strain WP2*uvrA*
- Dose Selection Criteria: Cytotoxicity
 - Basis of dose selection: Cytotoxicity in range-finding studies
 - Range finding studies: Examined minocycline exposure levels ranging from 0 to 5000 µg/plate, with and without S9. Exposures greater than 1.5 µg/plate produced excessive levels of cytotoxicity with most strains.
- Test Agent Stability: Acceptable
- Metabolic Activation System: Aroclor 1254-induced S9 from adult male SD rats
- Controls:
 - Vehicle: Water
 - Negative Controls: Vehicle
 - Positive Controls: 2-Nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), and methyl methanesulphonate (WP2*uvrA*) in absence of S9; 2-aminoanthracene in presence of S9
 - Comments: Controls were adequate
- Exposure Conditions:
 - Incubation times: 48 to 72 hours
 - Doses used in definitive study: 0.05-15 µg/plate
 - Study design: Plate assay
- Analysis:
 - No. slides/plates/replicates/animals analyzed: 2
 - Counting method: Dissecting microscope
 - Cytotoxic endpoints: > 50% reduction in number of spontaneous revertants on selective medium
 - Genetic toxicity endpoints: To be deemed positive, test article must cause a dose-related increase in the mean number of revertants of at least one tester strain over at least two concentrations

Results:

- Study Validity: Acceptable
- Study Outcome: Minocycline did not increase the incidence of revertants either in the presence or absence of S9. Appropriate results were obtained with the controls.

Conclusions: These data suggest minocycline is not mutagenic.

2.6.6.4.2 Study title: In vitro mammalian chromosome aberration test

Study No: AA57RE.341.BTL

Study Type: in vitro "chrom abs" assay in human peripheral blood lymphocytes

Amendment #, Volume # and Page #: NA

Conducting Laboratory: _____

Date of Study Initiation/completion: 23-APR-2002/21-AUG-2002

GLP Compliance: Yes

QA- Reports: Yes (X) No ()

Drug Lot Number: 329.55G047

Study Endpoint: Counting chromosomal aberrations (breaks, fragments, exchange figures) in metaphase cells

Methodology:

- Strains/Species/Cell line: Peripheral blood lymphocytes obtained from healthy, nonsmoking adults (one male, one female) were used
- Dose Selection Criteria: Cytotoxicity, as determined by reduced mitotic index
 - Basis of dose selection: Cytotoxicity in range-finding studies
 - Range finding studies: Examined concentrations of minocycline in culture medium ranging from 0.494-4940 µg/mL in assays involving 4 hour incubations with and without S9, as well as 20 hour incubations without S9
- Test Agent Stability: Acceptable
- Metabolic Activation System: Aroclor 1254-induced S9 from adult male SD rats
- Controls:
 - Vehicle: Water
 - Negative Controls: Vehicle
 - Positive Controls: Mitomycin C in absence of S9; cyclophosphamide in presence of S9
 - Comments: Controls were adequate
- Exposure Conditions:
 - Incubation times: 4 hours with and without S9; 20 hours without S9
 - Doses used in definitive study: 4 hr incubation in absence of S9: 50, 100, and 150 µg/mL. 4 hr incubation in presence of S9: 25, 50, and 125 µg/mL. 20 hr incubation in absence of S9: 6.25, 12.5, and 35 µg/mL.
 - Study design: Exposure for 4 hours with or without S9 followed by 16 hour resting period, or exposure for 20 hours without S9; in each case colcemid was added for final two hours prior to harvest. The cells were processed, fixed, placed on slides, stained, and microscopically examined.
- Analysis:
 - No. slides/plates/replicates/animals analyzed: 2 flasks
 - Counting method: Microscope
 - Cytotoxic endpoints: Reduced mitotic index (No. of mitotic figures x 100/500 cells)
 - Genetic toxicity endpoints: Increased numbers of cells that exhibited structural or numerical chromosome aberrations

Results:

- Study Validity: Acceptable
- Study Outcome: Minocycline did not increase the number of structural or numerical chromosome aberrations either in the presence or absence of S9. Appropriate results were obtained with the controls.

Conclusions: These data suggest minocycline is not clastogenic.

2.6.6.4.3 Study title: In vitro mammalian cell gene mutation test (CHO/HGPRT assay).

Key findings: Minocycline HCL was not mutagenic under the conditions of this assay.

Study No: AA57RE.782.BLT

Study Type: In vitro point mutation assay

Volume # and Page #: NA

Conducting Laboratory: _____

Date of Study Initiation: 12-APR-2004

GLP Compliance: Yes

QA Reports Yes (X) No ()

Drug, lot #, radiolabel, and % purity: Minocycline HCl, lot 329.55: _____

Formulation/vehicle: Dissolved/suspended in water

Methodology:

- Strains/Species/Cell line: CHO-K₁ cells
- Dose Selection Criteria: Cytotoxicity and physical compatibility (precipitate at conc. greater than 1500 µg/mL)
- Range finding studies: Examined concentrations from 0.5 to 5000 µg/mL, with and without S9
- Test Agent Stability: Chemical analyses of the test material formulations used in this study were apparently not performed
- Metabolic Activation System: Aroclor 1254-induced S9 (supernatant of the post-mitochondrial 9000 g fraction from adult male SD rats induced with a single injection of Aroclor-1254)
- Controls:
 - Vehicle: Water
 - Negative Controls: Vehicle
 - Positive Controls: Ethyl methanesulfonate in absence of S9;
Benzo(a)pyrene in presence of S9
 - Comments: Controls were adequate
- Exposure Conditions:
 - Incubation and sampling times: 5 hour exposure with or without S9
 - Doses used in definitive study: 25-125 µg/mL without S9; 25-250 µg/mL with S9
 - Study design: Following the exposure period, the cells were washed and grown in the presence of thioguanine (which screens for mutations that are unable to metabolize thioguanine)
- Analysis:
 - No. of replicates: Two
 - Counting method: Counted by eye

- Criteria for positive results: Considered positive if a concentration-related increase in mutant frequency was observed with at least two consecutive concentrations showing mutant frequencies of > 40 mutants per 10⁶ clonable cells.

Summary of individual study findings:

- Study Validity: Acceptable

Study Outcome: A concentration-response trend was not observed in either presence or absence of S9. Minocycline was not positive (genotoxic) under the criteria established for positive results. Appropriate results were obtained with the controls.

2.6.6.4.4 Study title: Mammalian erythrocyte micronucleus test

Study No: AA57RE.123.BTL

Study Type: Micronucleus assay

Amendment #, Volume # and Page #: NA

Conducting Laboratory: _____

Date of Study Initiation/completion: 23-APR-2002/15-SEP-2002

GLP Compliance: Yes

QA- Reports Yes (X) No ():

Drug Lot Number: 06853

Study Endpoint: Increase in percentage of micronucleated polychromatic erythrocytes

Methodology:

- Strains/Species/Cell line: ICR/mouse
- Dose Selection Criteria: Survival, clinical signs in dose-ranging study
- Basis of dose selection: Acceptable tolerance of acute doses that are large multiples of the clinical dose
- Range finding studies: Mice orally dosed with minocycline in water at exposure levels ranging from 1 to 2000 mg/kg.
- Test Agent Stability: Test material confirmed through chemical analysis to be approximately 93% pure and stable in solution throughout the experimental period
- Metabolic Activation System: NA
- Controls:
 - Vehicle: Water
 - Negative Controls: Vehicle
 - Positive Controls: Cyclophosphamide (50 mg/kg)
 - Comments: Controls were adequate
- Exposure Conditions:
 - Doses used in definitive study: 500, 1000, and 2000 mg/kg minocycline (gavage volume was 20 mL/kg)
 - Study design: In definitive micronucleus assay, 5 negative control mice per sex and 5 high-dose animals per sex were sacrificed at both 24 and 48 hours. For the low-dose, mid-dose, and positive control animals, 5 animals per sex were sacrificed at 24 hours only. Immediately

following sacrifice, the femurs were exposed, the bone marrow aspirated and centrifuged, the cells were suspended, and slides prepared, fixed, and stained. The slides were examined and 2000 polychromatic erythrocytes were scored for the presence of micronuclei (round, darkly staining nuclear fragments).

- Analysis:
 - No. slides/plates/replicates/animals analyzed: See above
 - Counting method: Microscope
 - Genetic toxicity endpoints: Significantly increased percentage of polychromatic erythrocytes with micronuclei

Results:

- Study Validity: Acceptable
- Study Outcome: No unscheduled deaths occurred, although lethargy and piloerection were observed at 1000 mg/kg and above. Minocycline did not increase the incidence of polychromatic erythrocytes with micronuclei. Appropriate results were obtained with the controls.

Conclusions: These data suggest minocycline is not clastogenic.

2.6.6.5 Carcinogenicity

To be evaluated post-approval.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

2.6.6.6.1 Study title: A study of fertility and early embryonic development to implantation of minocycline hydrochloride in rats

Key study findings: Male and female reproductive performances were unimpaired in this study, including no effects on the percentages of animals that copulated or became pregnant, or latency to mating. Spermatogenic endpoints were adversely affected at an exposure of 300 mg/kg/day, including a significant reduction in the mean number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and increased numbers of morphologically abnormal sperm cells. The latter effect was also observed at an exposure of 100 mg/kg/day. Morphological abnormalities observed in sperm samples from mid and/or high-dose group animals included absent heads, misshapen heads, and abnormal flagella. No differences in intrauterine survival were observed, including no effects on pre- or post-implantation losses or numbers of viable embryos. An exposure of 30 mg/kg/day was considered to be the NOAEL for male reproductive toxicology under the conditions of this study, due to effects on sperm morphology at 100 mg/kg/day. An exposure of 300 mg/kg/day was considered to be the NOAEL for female reproductive toxicology under the conditions of this study.

Study no.: — 450005

Volume #, and page #: NA

Conducting laboratory and location: _____

Date of study initiation: 19-MAR-2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Minocycline HCl, lot No. 329.55 _____

Methods

Doses: 0 (control), 30, 100, and 300 mg/kg/day, administered once daily.

Species/strain: Rat~~us~~ CD(SD)IGS BR; approx. 12 weeks old when paired.

Number/sex/group: 25

Route, formulation, volume, and infusion rate: Oral (gavage), 10 mL/kg. The test materials were prepared as suspensions/solutions of minocycline HCl at concentrations of 0 (vehicle control), 3, 10, or 30 mg/mL. Purified deionized water was used as the vehicle.

Satellite groups used for toxicokinetics: No

Study design: F0 males dosed beginning at least 28 days prior to pairing and continuing until one day prior to termination (59-64 days of treatment). F0 females dosed beginning at least 14 days prior to pairing until day 7 of gestation.

F0 females with no evidence of mating were dosed until day of euthanasia.

Animals paired 1:1 within a treatment group. Day of mating was gestation day 0.

Females placed with a proven male if no mating within 10 days. Females euthanized on gestational day 15.

Parameters and endpoints evaluated:

Clinical signs: Yes, twice daily

Body weight: Yes, twice weekly, plus females weighed on days 0, 3, 7, 10, 13, and 15 of gestation.

Food consumption: Yes

Estrous cycles: Vaginal lavages performed daily from 10 days prior to initiation of dosing until confirmation of mating (copulatory plug or presence of sperm), after which time females were individually housed.

Gross necropsy: Full necropsy of all F0 animals (male and female), including examination of uteri and ovaries. Numbers of embryos, resorptions, implantations, and corpora lutea were determined. Embryo viability was assessed.

Spermatogenic assessment: Immediately upon termination, the right epididymis was removed, weighed, processed, and a sperm sample was obtained through an incision in the distal portion of the right cauda epididymis and was assessed for sperm motility and morphology. The left testis and epididymis from each male was homogenized and evaluated for spermatid count and sperm production rate.

Organ weights: Yes; adrenals, brain, epididymides, kidneys, liver, ovaries, pituitary gland, testes, thyroids with parathyroids.

Histopathology: Thyroids from all animals and right testes from all F0 males.

Results

Mortality: No test-article-related deaths (one male in the low-dose group was found dead on study day 12; the cause of death could not be determined, but the death was judged to be incidental).

Clinical signs: Test article-related signs observed in high-dose animals at one-hour post-dosing included salivation, yellow, red, or clear material around the mouth or nose, and rales.

Body weight: No remarkable observations on mean weight or weight gain, including females during gestation.

Food consumption: No remarkable observations.

Toxicokinetics: NA

Necropsy/Organ weights: Dark-red discoloration of the thyroids, and yellow or green discoloration of the teeth and bones was observed in all treatment groups (both genders). The incidence of these observations tended to increase with increased dose. A statistically significant increase in mean thyroid weight was associated with increased dose in males; in females this effect was observed in the high-dose group only. This finding correlated with follicular cell hyperplasia. Dose-related, statistically significant, decreases in the mean epididymal weight were observed in males in the mid and high-dose groups.

Histopathology:

Thyroids: Minimal to moderate follicular cell hyperplasia of the thyroid was observed in both genders in all treatment groups; the severity tended to increase with dose. Similarly, the incidence of black pigment within follicular cells tended to increase with dose.

Testes: "Test article-related retention of spermatids was seen in 23 of 25 males in the 300 mg/kg/day group but was not detected at lower doses". The cause of this effect was unclear.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Reproductive performance: No treatment-related effects on either male or female reproductive performance, as indicated by the percentages of animals that successfully copulated (mating indices), became pregnant (fertility indices), or latency to mating.

Spermatogenic endpoints:

Sperm number (concentration): The mean epididymal sperm number (number of sperm cells per gram of epididymis) was statistically significantly lower in high dose males than

Drug, lot #, radiolabel, and % purity: Minocycline HCl, Lot No. 329.55. ~~_____~~
 pure (~~_____~~)

1

Formulation/vehicle: The test materials were prepared as suspensions/solutions of minocycline in water.

Methods:

Study overview:

| Group/Treatment | Exposure Level* (mg/kg/day) | Number of Females in Main Study | Number of Females in Toxicokinetic Group |
|-----------------|-----------------------------|---------------------------------|--|
| 1. Control | 0 | 25 | 0 |
| 2. Low Dose | 10 | 25 | 12 |
| 3. Mid Dose | 30 | 25 | 12 |
| 4. High Dose | 70 | 25 | 12 |

*Exposures are expressed in terms of minocycline base.

Species/strain: Rat ~~CD(SD)IGS BR~~

Doses employed: 0 (control), 10, 30, and 70 mg/kg/day minocycline (expressed in terms of the base content); dose volume was 10 mL/kg.

Route of administration: Oral (gavage) once daily

Study design: Virgin females (approx. 85 days old at time of pairing) were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Females only were dosed, beginning on day 6 of gestation and continuing through day 17 of gestation. Dams were killed on day 20 and C-sectioned. Males were untreated, of same strain as females, and approx. 20 weeks old at time of pairing.

Number/group: 25

Parameters and endpoints evaluated: Maternal survival and body weight.

Numbers of live, dead, and resorbed fetuses were determined. Live fetuses were weighed and examined for external, visceral, and skeletal anomalies. Blood samples were obtained from satellite animals on gestation days 6 and 17 for toxicokinetic purposes.

Results:

In-life (maternal) observations:

Maternal Mortality: None

Clinical signs: No remarkable observations

Maternal body weight gain: Significantly reduced body weight gain throughout the period of dosing at 30 mg/kg/day and above:

Mean maternal body weight gains over gestation days 6-18:

| Dose Group (mg/kg/day) | Body Weight Change (g) |
|---------------------------|------------------------------|
| 0 | 90±9.8 |
| 10 | 85±13.2 |
| 30 | 73±19.9** |
| 70 | 76±13.8** |

**p<0.01

Food consumption: No remarkable observations

Gross maternal pathology: Dark red discoloration of the thyroid was observed in all treatment groups, but not controls. No effects on organ weights. No other remarkable observations.

Toxicokinetics:

Toxicokinetic Parameters Calculated from Data Obtained on Gestation Day 17

| Dose Group (mg/kg/day) | AUC _{inf} (ng•hr/mL) | C _{max} (ng/mL) | T _{1/2} (hr) | T _{max} (hr) |
|---------------------------|----------------------------------|--------------------------|-----------------------|-----------------------|
| 10 | 29310 | 3470 | 2.32 | 2 |
| 30 | 89480 | 8650 | 5.11 | 4 |
| 70 | 223460 | 24950 | 3.70 | 4 |

Terminal and necroscopic evaluations (offspring):

Body weight of live fetuses: The mean fetal body weight (male/female combined) was significantly lower in all treatment groups:

| Dose Group (mg/kg/day) | Mean Fetal Body Weight, Male/Female combined (g) |
|---------------------------|---|
| 0 | 3.6±0.18 |
| 10 | 3.3±0.18** |
| 30 | 2.9±0.33** |
| 70 | 2.8±0.30** |

**p<0.01

Fetal viability: Although not statistically significant, the number of live fetuses at C-section (expressed as a percentage of implantations) was slightly reduced in the HD group (91.3% viability compared to 95.3% in the control group), apparently due to the slight increase in early resorptions in the HD group (see below).

No. of early resorptions: Although not statistically significant, the percentage of early resorptions was slightly higher in the HD group (7.8%, compared to 4.6% in the control group).

No. of late resorptions: No remarkable observations.

Post-implantation loss: Although not statistically significant, the mean post-implantation loss was slightly higher in the HD group (12.0%, compared to 4.6%, 6.0%, and 6.1% in the control, LD, and MD groups, respectively).

Gross non-skeletal anomalies: No remarkable external malformations or soft-tissue malformations or variations.

Skeletal anomalies: Bent limb bones (a malformation) were observed in 49 fetuses (from 6 litters) and in 7 fetuses (from 3 litters) in the MD and HD groups, respectively, but not in LD or control animals. Reduced skeletal ossification was observed in the MD and HD groups, including sternebrae unossified, bent ribs, 14th rudimentary ribs, and reduced ossification of the vertebral arches. The incidences of these skeletal variations were significantly higher in the MD and HD groups. No other remarkable observations.

2.6.6.6.3 Study title: A study of the effects of minocycline hydrochloride on embryo/fetal development in rabbits

Key study findings: When administered to female rabbits on days 7-20 of gestation, minocycline induced abortion in a minority of does, reduced maternal weight gain, gravid uterine weight, and mean fetal body weight, and induced skeletal malformations (bent limb bones). The NOAEL for maternal and fetal toxicity was 50 mg/kg/day.

Study no.: 450011

Volume #, and page #: NA

Conducting laboratory and location: _____

Date of study initiation: 12-APR-2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline HCl, Lot No. 329.55 _____

Formulation/vehicle: The test materials were prepared as suspensions/solutions of minocycline in water.

Methods:

Study overview:

| Group/Treatment | Exposure Level* (mg/kg/day) | Number of Females in Main Study | Number of Females in Toxicokinetic Group |
|-----------------|-----------------------------|---------------------------------|--|
| 1. Control | 0 | 22 | 0 |
| 2. Low Dose | 50 | 22 | 5 |
| 3. Mid Dose | 100 | 22 | 5 |
| 4. High Dose | 175 | 22 | 5 |

*Exposures are expressed in terms of minocycline base.

Species/strain: Rabbit/New Zealand white

Doses employed: 0 (control), 50, 100, and 175 mg/kg/day minocycline (expressed in terms of the base content); dose volume was 5 mL/kg.

Route of administration: Oral (gavage) once daily

Study design: Pregnant females (approx. 5.5 months old at time of receipt) were dosed beginning on day 7 of gestation and continuing through day 20 of gestation. Dams were killed on day 29 and C-sectioned.

Number/group: 22

Parameters and endpoints evaluated: Maternal survival and body weight.

Numbers of live, dead, and resorbed fetuses were determined. Live fetuses were weighed and examined for external, visceral, and skeletal anomalies. Blood samples were obtained from satellite animals on gestation days 7 and 20 for toxicokinetic purposes.

Results:

In-life (maternal) observations:

Maternal Mortality: No test-article induced mortality, but 1 LD, 1 MD, and 5 HD animals aborted spontaneously during gestation days 22-29, and were sacrificed.

Clinical signs: Treatment-related observations included decreased defecation and soft-stool.

Maternal body weight gain: Significantly reduced weight gain in HD animals over the treatment period:

Mean maternal body weight gains over gestation days 7-21:

| Dose Group (mg/kg/day) | Body Weight Change (g) |
|------------------------|------------------------|
| 0 | 429±201 |
| 50 | 351±166 |
| 100 | 335±223 |
| 175 | 148±297** |

**p<0.01

Food consumption: Significantly reduced in HD animals over various time intervals during the treatment period (e.g., over days 7-21, controls consumed 192±33 g/animal/day, while HD animals consumed 116±62 g/animal/day, p<0.01).

Gross maternal pathology: Dark red discoloration of the thyroid was observed in HD animals only. No effects on mean thyroid weight. The mean weight of the gravid uterus of HD animals was significantly lower than controls (417.3±158 g compared to 541±116 in controls, p<0.01). No other remarkable observations.

Toxicokinetics:

Toxicokinetic Parameters Calculated from Data Obtained on Gestation Day 20

| Dose Group (mg/kg/day) | AUC _{inf} (ng•hr/mL) | C _{max} (ng/mL) | T _{1/2} (hr) | T _{max} (hr) |
|------------------------|-------------------------------|--------------------------|-----------------------|-----------------------|
| 50 | 31799 | 3316 | 9.9 | 1 |
| 100 | 70179 | 7088 | 7.6 | 2 |
| 175 | 534811 | 17000 | 30.8 | 2 |

Terminal and necroscopic evaluations (offspring):

Body weight of live fetuses: The mean fetal body weight (male/female combined) was significantly lower in the HD group:

| Dose Group (mg/kg/day) | Mean Fetal Body Weight, Male/Female combined (g) |
|------------------------|--|
| 0 | 45.4±5.0 |
| 50 | 42.5±5.0 |
| 100 | 43.0±5.9 |
| 175 | 39.4±6.7** |

**p<0.01

Fetal viability: Although not statistically significant, the number of live fetuses at C-section (expressed as a percentage of implantations) was reduced in the HD group (88.3% viability compared to 98.5% in the control group).

No. of early resorptions: Although not statistically significant, the percentage of early resorptions was higher in the HD group (10.3%, compared to 1.0% in the control group).

No. of late resorptions: No remarkable observations.

Post-implantation loss: Although not statistically significant, the mean post-implantation loss was slightly higher in the HD group (13.1%, compared to 3.2%, 4.3%, and 4.0% in the control, LD, and MD groups, respectively).

Gross non-skeletal anomalies: No remarkable external malformations or variations, or soft-tissue malformations or variations.

Skeletal anomalies: Bent limb bones (a malformation) were observed in 1 fetus and in 9 fetuses in the MD and HD groups, respectively, but not in LD or control animals. The 9 fetuses with bent limbs in the HD group were all from the same litter. Bent limb bones were not observed in LD or control animals. No other skeletal malformations or variations were present in a manner that suggested a treatment-related effect.

Prenatal and postnatal development

2.6.6.6.5 Study title: A study of the effects of minocycline hydrochloride on pre- and postnatal development, including maternal function in rats

Key study findings: Minocycline was administered to pregnant female rats from day 6 of gestation through the period of lactation (postpartum day 20). Body weight gain was significantly reduced in high-dose F0 females during the period of treatment. There was no effect of treatment on the duration of the gestation period or on the number of dams with all pups dying postpartum. There was no effect on the mean number of pups born or the number of viable pups per litter. Postnatal survival was decreased with increasing exposure to minocycline. Gross external anomalies observed in F1 pups from the HD group (at higher incidence than in the control group) included smallness of size, malrotated forelimbs, and micromelia. No effects were observed on the physical development, behavior, learning ability, or reproduction of F1 pups, and there was no effect on gross appearance of F2 pups.

Study no.: — 450007

Volume #, and page #: NA

Conducting laboratory and location: _____

Date of study initiation: 09-JUN-2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Minocycline HCl, Lot No. 329.55 _____

Formulation/vehicle: The test materials were prepared as solutions/suspensions of minocycline HCl in water

Methods:

Species/strain: Rat ~~CD~~ CD(SD)IGS BR
 Number/sex/group: 25
 Doses employed: 0 (control), 5, 10, and 50 mg/kg/day
 Route of administration: Oral (gavage) once daily; dose volume of 10 mL/kg/day
 Satellite groups used for toxicokinetics: No
 Study design: Virgin females (approx. 85 days old at time of pairing) were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Females only were dosed, beginning on day 6 of gestation and continuing through lactation day 20 (postpartum day 20; a total of 36 to 38 doses). The test material was not administered to F0 males or to F1 animals. F0 females were sacrificed on day 21 postpartum. F1 animals of each gender (pups of F0 animals) were randomly selected for pairing with a non-sibling from the same treatment group for breeding of the F2 generation (cohabitated at approximately 90 days of age). The F1 breeder males were sacrificed at the conclusion of the cohabitation period. F1 breeder females were sacrificed on gestation day 20 and caesarean-sectioned. F1 animals were also assessed for effects on developmental landmarks, sensory function, behavior, learning, and memory.
 Parameters and endpoints evaluated: Body weights, food consumption, and clinical signs of all animals were monitored. F0 females were monitored for duration of gestation, litter size, pup viability, and nursing behavior. Gross necropsies were performed. F1 animals were evaluated on approximately day 70 postpartum for performance in a water-filled maze for overt coordination, swimming ability, learning, and memory. F1 males were monitored for the age of preputial separation and F1 females were monitored for the age of vaginal opening. F1 animals were observed for changes in mating behavior. F1 females were examined for numbers of corpora lutea, implantation sites, and viable fetuses. F2 fetuses were weighed and examined for gross external alterations.

Results

F₀ in-life: One high-dose (50 mg/kg/day) F0 female was found dead on gestation day 21; this death was considered to not be related to treatment (no deaths occurred in females in a prior study at 60 mg/kg/day; this death was presumably due to gavage error). No other unscheduled deaths. One MD F0 female lost her entire litter on lactation day 1 (the report doesn't explain, but she probably ate her pups). One F0 female each in the control, LD, and MD groups failed to deliver (upon necropsy these animals were found to have never been pregnant). All clinical signs, including the death, were considered incidental. Body weight gain was significantly reduced in high-dose F0 females during the portion of gestation in which treatment occurred (gestation days 6-20):

Mean maternal body weight gains over gestation days 6-20:

| Dose Group (mg/kg/day) | Body Weight Change (g) |
|---------------------------|---------------------------|
| 0 | 118±13.4 |

| | |
|----|------------|
| 5 | 121±13.9 |
| 10 | 114±11.8 |
| 50 | 105±14.1** |

**p<0.01

Mean body weights and weight gains were not affected by treatment during the period of lactation. Food consumption by F0 females did not differ between groups during gestation, but tended to be slightly reduced in HD F0 females during lactation. There was no effect of treatment on the duration of the gestation period or on the number of dams with all pups dying postpartum. The majority of F0 females that received minocycline exhibited dark red discoloration of the thyroid, and some HD F0 females had greenish discoloration of the teeth. Mean absolute weight of the thyroid was significantly increased in HD F0 females (mean absolute weight of 0.0258±0.0048 g in HD animals compared to 0.0204±0.0038 g in controls, p<0.01).

F₁ litter data: There was no effect on the mean number of pups born or the number of viable pups per litter (intrauterine survival). Postnatal survival was decreased with increasing exposure to minocycline (5, 6, 22, and 31 pups found dead, and 4, 1, 22, and 13 pups disappeared (were cannibalized) in the control, LD, MD, and HD groups, respectively). Gross external anomalies observed in F1 pups from the HD group (at higher incidence than in the control group) included smallness of size (11 pups from 6 litters), malrotated forelimbs (14 pups from 6 litters), and micromelia (short limbs; 22 pups from 14 litters). Mean pup weight tended to be slightly reduced in the HD group during the period of lactation. During necropsy of F1 animals, two males and two females in the HD group exhibited yellow discoloration of teeth of bone.

F₁ physical development: No remarkable observations (no effects on time to balanopreputial separation or vaginal opening).

F₁ behavioral evaluation: No remarkable observations (no effects on acoustic startle response, locomotor activity, or performance in Biel maze swimming trials).

F₁ reproduction: No remarkable observations (no effects on reproductive performance, mating indices, maternal body weight gain, gravid uterine weights, implantation, or viability or morphology of F2 fetuses).

F₂ findings: No remarkable observations.

2.6.6.7 Local tolerance

Not applicable.

2.6.6.8 Special toxicology studies

None.

2.6.6.9 Discussion and Conclusions

Minocycline was reasonably well tolerated in general toxicology studies. In 90 day repeat-dose toxicology studies conducted in mice, rats, and monkeys, little toxicity was observed at exposures comparable to the clinical level of exposure. The primary toxicological target organ for minocycline in all species appears to be the thyroid, with treatment-related effects on the thyroid manifesting (at sufficient levels of exposure) as grossly visible enlargement, increased mean weight, dark red discoloration, increased colloid content, accumulation of brown pigment in the follicular cells, follicular cell hypertrophy, and elevated plasma levels of T4 and TSH. These effects are of a minimal to mild severity in most instances, and do not appear to be toxicologically relevant at levels of exposure close to those observed clinically. Minocycline was negative in a battery of genetic toxicology studies, and is apparently not genotoxic. Minocycline may be capable of impairing fertility in human males. When administered to male rats minocycline adversely affected spermatogenic endpoints, including a significant reduction in the mean number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and increased numbers of morphologically abnormal sperm cells. Morphological abnormalities observed in sperm samples included absent heads, misshapen heads, and abnormal flagella. However, male and female reproductive performances were not impaired in that study; there were no effects on the percentages of animals that copulated or became pregnant, or latency to mating. When assessed for teratogenic effects in rats, minocycline reduced the mean fetal weight and induced skeletal malformations (bent limb bones) and skeletal variations (reduced skeletal ossification). When administered to female rabbits during the period of organogenesis, minocycline induced abortion in a minority of does, reduced maternal weight gain, gravid uterine weight, and mean fetal body weight, and induced skeletal malformations (bent limb bones). When assessed for effects on pre- and postnatal development, including maternal function, in rats, minocycline had no effect on the duration of the gestation period or on the number of dams with all pups dying postpartum. There was no effect on the number of viable pups per litter, although postnatal survival was decreased with increasing exposure to minocycline. Gross external anomalies observed in F1 pups included smallness of size, malrotated forelimbs, and micromelia. No effects were observed on the physical development (time to balanopreputial separation or vaginal opening), behavior, learning ability, or reproduction of F1 pups, and there was no effect on gross appearance of F2 pups. Minocycline was evaluated in a battery of safety pharmacology studies. Minocycline had no effect on behavior, psychological state, arterial blood pressure, heart rate, the ECG, or (at clinically relevant levels of exposure) respiration.

The excipients in the product are all associated with substantial clinical use by the oral route, and should be considered to be qualified for the proposed new use.

The clinical formulation of the drug product and the individual components of the product have been adequately evaluated for safety and the database supports the safety of the proposed use of the product.

2.6.6.10 Tables and Figures

Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Summary of Systemic Exposure Data at the NOAEL in Selected Nonclinical Studies:

| Study Type | NOAEL ¹ (mg/kg/day) | AUC ² at NOAEL (ng·hr/mL) | AUC Ratio ³ |
|------------------------------------|-----------------------------------|---|----------------------------|
| 13 Week Repeat-Dose Oral Mouse | 50 | Males: 23577 Females: 23829 | Males: 0.7 Females: 0.7 |
| 13 Week Repeat-Dose Oral Rat | 50 | Males: 245360 Females: 245340 | Males: 7 Females: 7 |
| 13 Week Repeat-Dose Oral Monkey | 10 | Males: 90070 Females: 91420 | Males: 3 Females: 3 |
| Male and Female Fertility, Rat | 30 in males, 300 in females | Males: 147204 ^{4,5} Females: 1472040 ⁴ | Males: 4 Females: 40 |
| Teratology, Rat | 10 | 29310 | 0.9 |
| Teratology, Rabbit | 50 | 31799 | 1 |
| Perinatal Development, Rat | 5 | 14655 | 0.4 |

¹No-Adverse-Effect-Level; level at which no substantial toxicity was observed.

²AUC = "Area under curve" when mean plasma-concentration is plotted against time.

³AUC ratio refers to $AUC_{\text{nonclinical}}/AUC_{\text{clinical}}$; AUC_{clinical} refers to the AUC values observed in patients under conditions of maximum exposure (estimated at 33320 ng·hr/mL in both males and females). AUC ratios can be considered to be "safety factors", or margins of safety.

⁴Calculated via extrapolation.

⁵The NOAEL for male reproductive performance (ability to mate and impregnate) was 300 mg/kg/day, but the NOAEL for morphological effects on sperm cells was 30 mg/kg/day.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The product is approvable with respect to nonclinical concerns.

Unresolved toxicology issues (if any): The carcinogenicity of minocycline is to be assessed post-approval.

Recommendations: The product is approvable with respect to nonclinical concerns.

Suggested labeling:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:

Long-term animal studies have not been performed to evaluate the carcinogenic potential of minocycline. A structurally related compound, oxytetracycline, was found to produce adrenal and pituitary tumors in rats.

Minocycline was not mutagenic *in vitro* in a bacterial reverse mutation assay (Ames test) or in a CHO/HGPRT mammalian cell assay in the presence or absence of metabolic activation. Minocycline was not clastogenic *in vitro* in human peripheral blood lymphocytes or *in vivo* in a mouse micronucleus test.

Male and female reproductive performance in rats was unaffected by oral doses of minocycline of up to 300 mg/kg/day (which resulted in up to approximately 40 times the level of systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets). However, oral administration of 100 or 300 mg/kg/day of minocycline to male rats (resulting in approximately 15 to 40 times the level of systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets) adversely affected spermatogenesis. Effects observed at 300 mg/kg/day included a reduced number of sperm cells per gram of epididymis, an apparent reduction in the percentage of sperm that were motile, and (at both 100 and 300 mg/kg/day) increased numbers of morphologically abnormal sperm cells. Morphological abnormalities observed in sperm samples included absent heads, misshapen heads, and abnormal flagella. SOLODYN[®] tablets should not be used by individuals who are attempting to conceive a child.

Pregnancy:

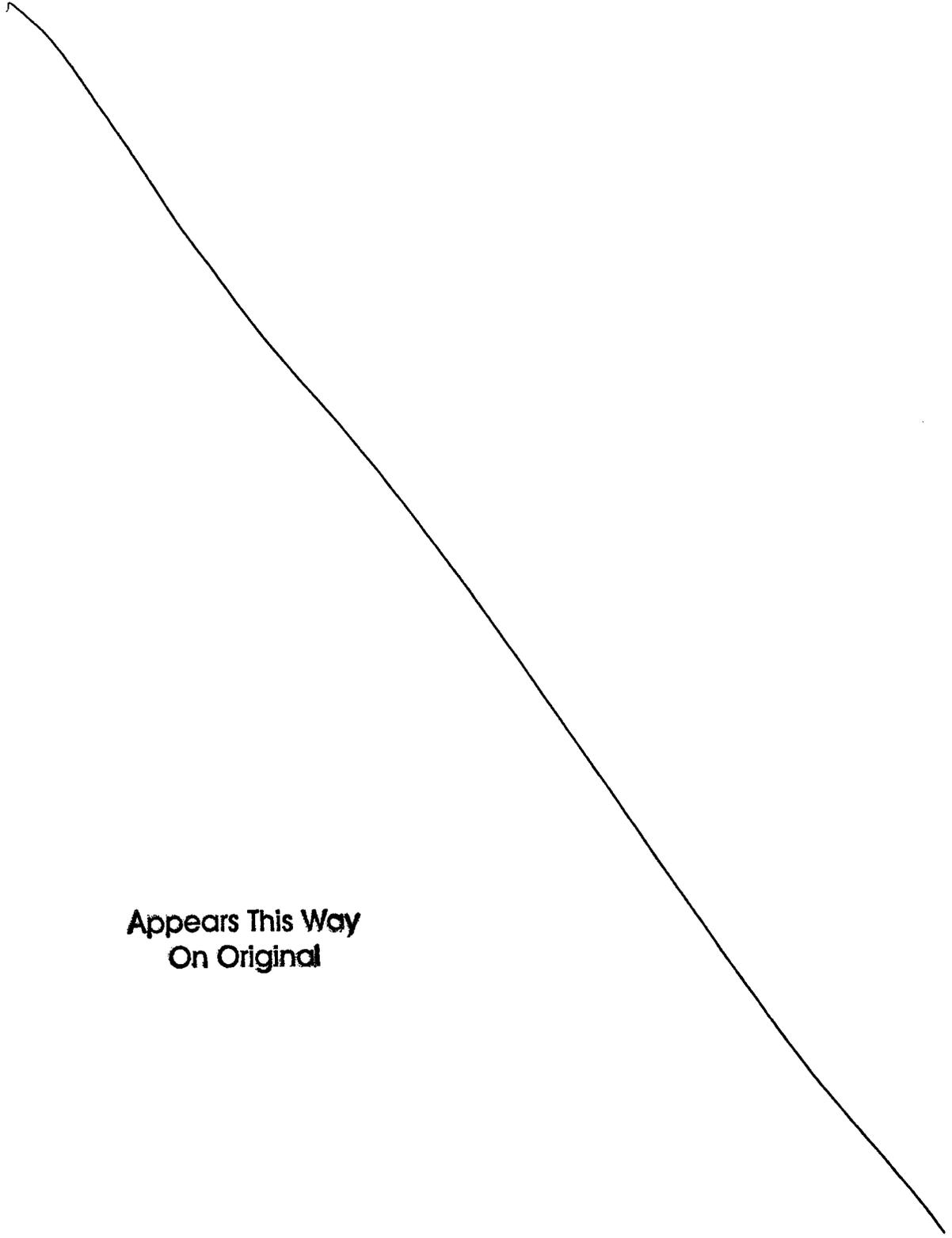
Teratogenic Effects: Pregnancy Category D. See WARNINGS. Tetracycline-class antibiotics, such as minocycline, are known to be capable of inducing harm to a fetus when administered to a pregnant woman. Minocycline induced skeletal malformations (bent limb bones) in fetuses when administered to pregnant rats and rabbits in doses of 30 mg/kg/day and 100 mg/kg/day, respectively (resulting in approximately 3 times and 2 times, respectively, the systemic exposure to minocycline observed in patients as a result of use of SOLODYN[®] tablets). Reduced mean fetal body weight was observed in studies in which minocycline was administered to pregnant rats at a dose of 10 mg/kg/day (which resulted in approximately the same level of systemic exposure to minocycline as that observed in patients who use SOLODYN[®] tablets). SOLODYN[®] tablets should not be used during pregnancy.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS: NONE



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/s/

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