

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-430**

**PHARMACOLOGY REVIEW(S)**

**Note: This review is an updated version and is to replace Review #1. The following sections have been updated to reflect new information: Pharmacology, Carcinogenesis, and Suggested Labeling.**

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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: 21-430  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 11/23/05  
PRODUCT: Thalomid®/ Thalidomide  
INTENDED CLINICAL POPULATION: "Patients with multiple myeloma"  
  
SPONSOR: Celgene Corporation  
DOCUMENTS REVIEWED: Electronic submissions of Dec 22, 2003 and Nov 23, 2005  
REVIEW DIVISION: Division of Drug Oncology Products  
PHARM/TOX REVIEWER: Haleh Saber-Mahloogi, Ph.D.  
PHARM/TOX SUPERVISOR: David E Morse, Ph.D.  
DIVISION DIRECTOR: Robert Justice, M.D.  
PROJECT MANAGER: Carl Huntley, R.Ph., MBA

Date of review submission to Division File System (DFS): May 22, 2006

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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

- A. Recommendation on approvability: There are no Pharmacology/Toxicology issues which preclude approval of the requested product indication.
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: See “Overall Conclusions and Recommendations”.

### **II. Summary of nonclinical findings**

- A. Brief overview of nonclinical findings: see section 2.6.2.2 for review of nonclinical pharmacology and section 2.6.6.6 for review of reproductive toxicity. The reproductive toxicity studies were reviewed under IND 48,177; a copy of which is included in this NDA review. The carcinogenicity studies were reviewed under NDA 20,785.
- B. Pharmacologic activity: immuno-modulatory and anti-angiogenic activities
- C. Nonclinical safety issues relevant to clinical use: See the original NDA

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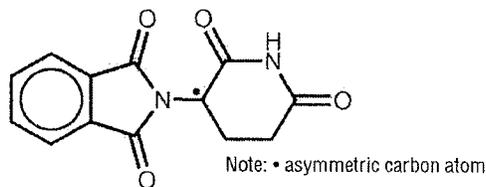
## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21,430  
**Review number:** 2  
**Sequence number/date/type of submission:** 000/Nov 23, 2005/sNDA Type 6  
**Information to sponsor:** Yes ( ) No (X)  
**Sponsor and/or agent:** Celgene Corporation  
7 Powder Horn Dr.  
Warren, NJ 07059  
**Manufacturer for drug substance:** See the original NDA  
**Reviewer name:** Haleh Saber-Mahloogi, Ph.D.  
**Division name:** Division of Drug Oncology Products  
**Review completion date:** May 11, 2006

#### Drug:

**Trade name:** Thalomid®  
**Generic name:** Thalidomide  
**Code name:** not provided  
**Chemical name:** (alpha)-(N-phthalimido) glutarimide  
2-(2,6-dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione  
**CAS registry number:** 50-35-1  
**Molecular formula/molecular weight:** C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>/ 258.2  
**Structure:**



#### Relevant INDs/NDAs/DMFs:

IND \_\_\_\_\_  
IND 48177: treatment of erythema nodosum leprosum  
IND 49481: \_\_\_\_\_  
IND \_\_\_\_\_  
NDA 20785: treatment of erythema nodosum leprosum and maintenance therapy

**Drug class:** immunomodulatory, anti-angiogenic

**Intended clinical population:** "patients with multiple myeloma" \_\_\_\_\_

**Clinical formulation:** capsules; see the original NDA

**Route of administration:** Oral

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

**Studies reviewed within this submission:**

Pharmacology

- Articles on the pharmacology of thalidomide. See the references under section 2.6.2.2.
- Celgene Report # 5071-180: Lenalidomide inhibits angiogenesis in vitro and reduces lung metastasis of mouse melanoma cells in an animal model.

*Note: Only the study pertaining to anti-angiogenic properties of thalidomide, submitted within this report was reviewed.*

**Studies reviewed by other divisions:**

The following studies were reviewed under NDA 20,785 by the Division of Anti-Infective Drug Products:

- 104-Week Oncogenicity Study of Thalidomide in Rats
- 104-Week Oncogenicity Study of Thalidomide in Mice

The following studies were reviewed under IND 48,177 by the Division of Anti-infective Drug products:

- Oral (Stomach Tube) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of (+) Thalidomide in Rabbits, Including a Postnatal Reproductive Evaluation (Segment III)
- Oral (Stomach Tube) Fertility and General Reproduction Toxicity Study of (±) Thalidomide in Rabbits (Segment I)

**Studies not reviewed within this submission:**

No studies submitted.

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## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Thalomid® (thalidomide) is an immuno-modulator approved for the treatment of erythema nodosum leprosum in 1998, NDA # 20-785, at doses of 100- 400 mg orally once daily at bedtime. Thalidomide is a racemic mixture of equal quantities of R- and S- stereoisomers.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action:

**Immuno-modulation:**

Thalidomide can both reduce (1;2) and increase the levels of the cytokine tumor necrosis factor (TNF- $\alpha$ ) (3). Leprosy patients with erythema nodosum leprosum (an inflammatory manifestation) treated with thalidomide, experience a reduction of serum TNF- $\alpha$  levels with a concomitant abrogation of clinical symptoms (4). In patients with tuberculosis, with or without

HIV infection, thalidomide reduced plasma TNF- $\alpha$  protein levels and leukocyte TNF- $\alpha$  mRNA levels (5). In addition, it was shown by Davies et al. that thalidomide at 5  $\mu\text{g/mL}$  (equivalent to approximately 19  $\mu\text{M}$ ) can act as a co-stimulatory signal to T cells by significantly increasing interferon  $\gamma$  (IFN- $\gamma$ ) and interleukin 2 (IL-2) production in culture, although analogs of thalidomide showed much higher activity. This concentration of thalidomide induced natural killer (NK) cell mediated lysis of multiple myeloma (MM) cells in culture(6). Five patients received thalidomide at 200 to 800 mg/day. The 3 patients that responded to therapy had 2.1 to 5.6 fold increases in the CD56+ NK cells, compared to 0.5 and 1.2 fold increases in the 2 patients who failed to respond (6). In patients with advanced HIV infection, aphthous ulceration of the mouth and oropharynx can become extensive. Treatment of these patients with 200 mg/day of thalidomide for 4 weeks resulted in an increase in both the plasma concentrations of TNF- $\alpha$  and soluble TNF- $\alpha$  receptors; 16/29 patients had complete healing of their ulcers after 4 weeks compared to 2/28 patients in the placebo group (3).

Other groups have shown little or no effect of thalidomide (100 mg/kg or 600 mg/m<sup>2</sup> administered twice orally, with 12 hr interval for a total of 200 mg/kg or 1200 mg/m<sup>2</sup>) on circulating levels of TNF- $\alpha$  in animal studies in which male Wistar rats were induced to produce TNF- $\alpha$  by treatment with LPS (7).

Thalidomide has also been shown to change levels of IL-10 and IL-12 (see below).

#### **Anti-inflammatory activity:**

Anti-inflammatory activities of thalidomide could be explained in part by thalidomide-induced immuno-modulation, e.g.  $\downarrow$ TNF- $\alpha$  (see above), and  $\uparrow$ IL-10 (see below). In addition, thalidomide was shown to inhibit the lipopolysaccharide (LPS)-mediated induction of Cox-2 and prostaglandin biosynthesis in murine macrophage cells in culture (8). The Cox-2 and PG inhibition took place at concentrations of thalidomide tested between 10 and 50  $\mu\text{M}$ . The reduction was statistically significant for PGE2 production at all thalidomide concentrations.

IL-10 is an inhibitor of monocyte/macrophage activation, blocking the expression of TNF- $\alpha$  and other pro-inflammatory mediators (9). Since thalidomide has been shown to suppress the production of TNF- $\alpha$  in stimulated human monocytes (2), thalidomide and its analogs were investigated for effects on the production of IL-10. It was shown that the LPS-induced IL-10 levels were increased by thalidomide ( $p < 0.05$ ), while the LPS-induced IL-12 and TNF- $\alpha$  were significantly inhibited (10). In another study, thalidomide was shown to inhibit IL-12 production by blood mononuclear cells stimulated with either heat-killed SAC or LPS (11). In the same PBMC cultures, thalidomide inhibited production of both TNF- $\alpha$  and IL-10. The suppression of IL-12 production by thalidomide was suggested to provide a mechanism for the immunosuppressive activity of the drug, independent or synergistic with its anti-TNF- $\alpha$  effects.

In a Wistar rat study, thalidomide administration slightly increased the plasma levels of IL-10, in LPS-treated rats (7).

#### **Anti-angiogenic effect:**

Orally administered thalidomide at 200 mg/kg or 2400 mg/m<sup>2</sup> inhibited angiogenesis induced by basic fibroblast growth factor (bFGF) in the rabbit cornea micropocket assay by 30 to 51% (12).

Although not clear from the article, it appears that thalidomide was administered from Day 2 to Day 12 of the study and Day 8 measurements were used for comparison between groups. This effect appears to be independent of the effect on TNF- $\alpha$ .

In a study by Gupta et al (13), it was shown that VEGF was secreted by most MM cell lines studied. VEGF and IL-6 (an MM growth and survival factor) secretion increased significantly in cultures of MM cells adherent to bone marrow stromal cells (BMSCs), due to enhanced secretion of these cytokines from BMSCs in a predominant paracrine fashion. Thalidomide at 100  $\mu$ M reduced VEGF and IL-6 secretion in co-cultures of BMSCs and MM cells. Thalidomide at 100  $\mu$ M also resulted in reduced VEGF secretion in MM cell lines, and VEGF and IL-6 secretion in BMSCs alone. The effect was larger in the co-cultures. There are reports indicating that thalidomide does not change or increase the levels of IL-6 (7).

Other studies show that the anti-tumor activity of thalidomide in MM is only partially attributed to its anti-angiogenic activity. In the article by Singhal et al (14), thalidomide produced clinical response in 32% of patients whose disease was refractory to conventional therapy, however, there was no correlation between bone marrow angiogenesis and response to treatment.

In a study by Gelati et al (15), thalidomide at approximately 4  $\mu$ M, resulted in reduction of VEGF-induced proliferation of HUVEC cells, only when HUVEC cells were plated on vitronectin. Reductions in HUVEC cell proliferation of 20-30% was reported by this group.

Thalidomide was shown to inhibit angiogenesis in a human umbilical artery explant model in vitro. Below is the summary of the report sponsored by Celgene:

Report# 5071-180

Study date: August 28, 2000- December 18, 2003

Study site: Celgene Corporation, 7 Powder Horn Drive, Warren, NJ

Title of the report: Lenalidomide inhibits angiogenesis in vitro and reduces lung metastasis of mouse melanoma cells in an animal model.

*Only one study in this report was reviewed, see below.*

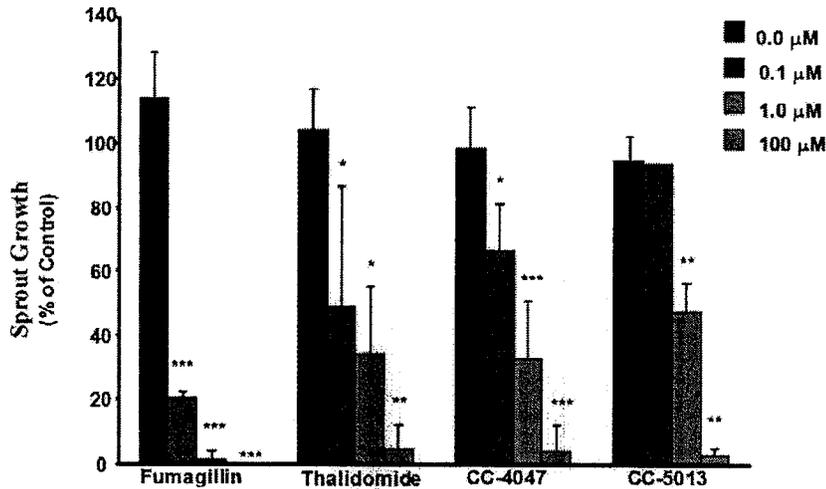
Title of the study: Thalidomide and IMiDs (Immunomodulatory Drugs) inhibit the formation of a sprout from human explants

Method: in vitro rat aortic ring model is an assay used to measure the angiogenic effects of a substance. The sponsor optimized this assay using blood vessel fragments from human umbilical arteries. The effects of thalidomide, lenalidomide (an analog of thalidomide) and CC-4047 were tested on the formation of sprouts from human blood vessels.

Results:

Untreated and DMSO-treated samples showed significant outgrowth of sprouts around the fragments after three weeks of growth in a culture. The addition of thalidomide, CC-4047 and lenalidomide (CC-5013) inhibited the formation of microvessel outgrowth in a dose-dependent manner when compared with either untreated or DMSO-treated samples.

### Human Umbilical Cord Vessel Rings Assay



Data are presented as mean  $\pm$  SD. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 vs control (DMSO at 0.0  $\mu$ M), 1-way ANOVA with Newman-Keuls Multiple Comparison Test.

Graph provided by the sponsor.

#### Anti-proliferative activity:

Effects of thalidomide and its analogs were studied on DNA synthesis by MM cell line and freshly derived patient MM cells (16). When studies were conducted in MM cell lines, only 15% to 20% inhibition in MM.1S and Sultan cells were observed in cultures at high concentrations (100  $\mu$ M) of thalidomide, in contrast to the analogs. Analogs of thalidomide resulted in 50% inhibition of proliferation in MM.1S and Sultan cells at concentrations of 0.1  $\mu$ M (IMiD1 analog) or 1.0  $\mu$ M (IMiD2 and IMiD3 analogs). Similar to what was seen in culture, study in patients MM cells showed that the inhibitory effect of thalidomide, even at 100  $\mu$ mol/L was not significant. Thalidomide had little or no effect on inhibition of DNA synthesis of MM cells resistant to other therapies. In the IL-6-stimulated MM.1S MM cell line, thalidomide had no effect on the activated MAPK cascade (no apoptotic activity through  $\downarrow$ MAPK phosphorylation). In summary, compared to its analogs, thalidomide had minimal apoptotic activity.

Confirming the above discussion, Davies et al. (6) referring to the article by Hideshima et al, stated that “the growth arrest and apoptotic activity of thalidomide are observed only at relatively high concentrations not readily achievable in plasma”.

## Reference List

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- (10) Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. *J Immunol* 1999; 163(1):380-386.
- (11) Moller DR, Wysocka M, Greenlee BM, Ma X, Wahl L, Flockhart DA et al. Inhibition of IL-12 production by thalidomide. *J Immunol* 1997; 159(10):5157-5161.
- (12) D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci U S A* 1994; 91(9):4082-4085.
- (13) Gupta D, Treon SP, Shima Y, Hideshima T, Podar K, Tai YT et al. Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. *Leukemia* 2001; 15(12):1950-1961.

- (14) Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med 1999; 341(21):1565-1571.
- (15) Gelati M, Corsini E, Frigerio S, Pollo B, Broggi G, Croci D et al. Effects of thalidomide on parameters involved in angiogenesis. J Neuro-Oncology 2003; 64: 193-201.
- (16) Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood 2000; 96(9):2943-2950.

Drug activity related to proposed indication:

Immuno-modulatory and anti-angiogenic activities.

**2.6.2.3 Secondary pharmacodynamics**

No studies submitted.

**2.6.2.4 Safety pharmacology**

No studies submitted.

**2.6.2.5 Pharmacodynamic drug interactions**

No studies submitted.

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**2.6.3 PHARMACOLOGY TABULATED SUMMARY**

No studies submitted.

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**2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

No studies submitted.

**2.6.4.1 Brief summary**

No studies submitted.

**2.6.4.2 Methods of Analysis**

No studies submitted.

**2.6.4.3 Absorption**

No studies submitted.

**2.6.4.4 Distribution**

No studies submitted.

**2.6.4.5 Metabolism**

No studies submitted.

**2.6.4.6 Excretion**

No studies submitted.

**2.6.4.7 Pharmacokinetic drug interactions**

No studies submitted.

**2.6.4.8 Other Pharmacokinetic Studies**

No studies submitted.

**2.6.4.9 Discussion and Conclusions**

No studies submitted.

**2.6.4.10 Tables and figures to include comparative TK summary**

No studies submitted.

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**2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

No studies submitted.

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**2.6.6 TOXICOLOGY**

No studies submitted.

**2.6.6.1 Overall toxicology summary**

General toxicology: No studies submitted.

Genetic toxicology: No studies submitted.

Carcinogenicity: The carcinogenicity studies have been reviewed by the Division of Anti-Infective Drug Products, review date of 2/23/04, as part of NDA # 20-785.

The following has been proposed by the review division for the carcinogenicity section of the labeling:

Two-year carcinogenicity studies were conducted in male and female rats and mice. No compound-related tumorigenic effects were observed at the highest dose levels of 3,000 mg/kg/day to male and female mice (38-fold greater than the highest recommended daily human dose of 400 mg based upon body surface area [BSA]), 3,000 mg/kg/day to female rats (75-fold the maximum human dose based upon BSA), and 300 mg/kg/day to male rats (7.5-fold the maximum human dose based upon BSA).

Reproductive toxicology: The reproductive toxicity studies consisted of Segments 1 and 3. These studies were reviewed by the Division of Anti-Infective Drug Products as consultation for the Division of Special Pathogen and Immunologic Drug Products. A copy of the review is included under Reproductive and Developmental Toxicity, section 2.6.6.6.

Special toxicology: No studies submitted.

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**2.6.6.2 Single-dose toxicity**

No studies submitted.

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**2.6.6.3 Repeat-dose toxicity**

No studies submitted.

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**2.6.6.4 Genetic toxicology**

No studies submitted.

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**2.6.6.5 Carcinogenicity**

Carcinogenicity: The carcinogenicity studies have been reviewed by the Division of Anti-Infective Drug Products, review date of 2/23/04, as part of NDA # 20-785.

The following has been proposed by the review division for the carcinogenicity section of the labeling:

Two-year carcinogenicity studies were conducted in male and female rats and mice. No compound-related tumorigenic effects were observed at the highest dose levels of 3,000 mg/kg/day to male and female mice (38-fold greater than the highest recommended daily human dose of 400 mg based upon body surface area [BSA]), 3,000 mg/kg/day to female rats (75-fold the maximum human dose based upon BSA), and 300 mg/kg/day to male rats (7.5-fold the maximum human dose based upon BSA).

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**2.6.6.6 Reproductive and developmental toxicology**

The following is a copy of the reproductive toxicity studies submitted to IND 48,177 and reviewed by the Division of Anti-Infective Drug Products.

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**Oral (Stomach Tube) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of (+) Thalidomide in Rabbits, Including a Postnatal Reproductive Evaluation (Segment III)** (Protocol 2103-001) Submitted to IND 48,177-086

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Report dated 8/18/03, U.S. and Japanese GLP, signed QA statement present

**Animals:** Timed-mated New Zealand White rabbits [Hra:(NZW)SPF], 2.8-3.8 kg at time of study assignment, 25/treatment group, plus 6 satellite does per treatment for blood and milk samples; presumed pregnant does were individually housed, offspring were group housed until PND 49, then they were individually housed.

**Diet:** \_\_\_\_\_ was offered daily. Approximately 150-155 g/day was offered to F0 females up to GD 6, then 180-185 g/day was offered for the duration of pregnancy and 230-235 g was offered during lactation. While F1 rabbits were group housed, they were fed *ad libitum*. Afterward, 150-155 g/day was offered to males and nonpregnant females and 180-185 g/day was offered to pregnant females. Tap water that was further purified by reverse osmosis and containing chlorine as a bacteriostat was available *ad libitum* to all rabbits.

**Drug Dose and Route of Administration:** Thalidomide (Batch #574-574-00-015; purity 99.8%) was administered daily to F0 females by oral gavage at doses 30, 150, and 500 mg/kg/day. The vehicle control group received aqueous 1% carboxymethylcellulose. Doses were set based upon the results of a range-finding study conducted at the laboratory.

**Length and Conduct of Study:** Presumed pregnant does received thalidomide or vehicle from GD 18 through PND 28 (or GD 33 for animals that did not deliver a litter).

F0 does were observed for viability at least twice daily and clinical signs were recorded before dosing and about one hour after dosing. Body weights were measured on GDs 0, 7, 10, 12, 15, daily during dosing, and at sacrifice. Food consumption was recorded daily until PND 21.

Does were observed during parturition for possible adverse effects and the duration of gestation, litter size, pup viability indices, and lactation indices were assessed. Maternal behavior was evaluated on PNDs 4, 7, 14, 21, and 28. Litters were not culled out of concern that bias could be introduced.

Blood and milk samples were collected from the drug-treated satellite F0 does once each week during the lactation period approximately 3 hr after dosing (the time of expected C<sub>max</sub> based upon previous studies with rabbits). Plasma and milk samples were diluted with \_\_\_\_\_, then stored frozen until analysis ( \_\_\_\_\_ ) using a validated HPLC/MS/MS method. The concentration ranges for measuring thalidomide in the blood and milk samples were 1-1000 ng/ml and 5-2500 ng/ml, respectively.

F1 pups were checked for viability twice daily. During the lactation period, the pups in each litter were counted once daily from the time of birth and observed for

clinical signs each day beginning on PND 4. During the postweaning period, clinical observations were recorded weekly and food consumption was recorded daily. Body weights were measured at birth, on PNDs 4 and 7, then weekly thereafter. F1 females were examined for vaginal patency starting on PND 36 and males were evaluated for sexual maturation beginning at approximately 3 months of age.

F0 does assigned to the main study were sacrificed after the lactation period was complete, or following abortion or death of all pups. Aborted fetuses, fetuses remaining in the uterus, and dead pups were examined to the extent possible. Pups that died before the initial examination of the entire litter for viability were evaluated to determine their vital status at birth by removing the lungs and immersing them in water (lungs from stillborn pups sink). The F0 does that did not deliver a litter were sacrificed 33 days following insemination and the uteri were stained with ammonium sulfide to confirm a lack of implantation sites. All does assigned to the main study underwent gross necropsy and the number and distribution of implantation sites were recorded.

F1 pups that were observed with splayleg were removed from the study at 29, 40-46, or 51 days of age. They were photographed and videotaped prior to sacrifice. One control pup with limb splay and 3/gender with limb splay from the high dose group were perfused to preserve them for histopathological/neuropathological evaluation. At the same time, 3 apparently normal pups/gender from the vehicle group were selected and also sacrificed and perfused. The perfused carcasses were sent to Consultants in \_\_\_\_\_ where they were x-rayed and multiple sections of the brain, spinal cord, spinal nerve roots, dorsal root ganglia, peripheral nerves, and skeletal muscle from each rabbit were examined microscopically.

F1 pups from the mid and high dose groups that were not chosen for learning/memory and/or reproductive evaluation were sacrificed at 49 days of age and grossly necropsied. Their hearts were examined using a variation of the Staples microdissection technique and their brains were examined *in situ* after a cross section was cut between the parietal and frontal bones. Those from the control and low dose thalidomide groups not chosen for additional evaluation were sacrificed at 29 days of age and discarded without further evaluation.

On PND 49, F1 offspring were randomly selected for continued evaluation. When possible, 1/sex was selected from each litter in the vehicle and low dose groups and 2/sex were selected from each litter in the mid and high dose groups. Up to 8 F1 rabbits/sex per dose group were selected for 1 of 2 evaluations of learning and memory using eyeblink conditioning (the delay paradigm and the trace paradigm). Prior to the initiation of this behavioral testing, the animals were conditioned to being restrained and having equipment attached to their heads for 15 minutes on the first day, 30 minutes on the second day, 2 days of rest, then 60 minutes on a third day while baseline data (without stimuli) were collected. After the conditioning period, testing sessions consisting of 60 trials with 60 seconds between each trial were initiated. A tone (the conditioned stimulus) was paired with a corneal air puff (the unconditioned stimulus) and the movement of the nictitating membrane (response) was recorded. Initially, the response correlated with the air puff, but as the animals learned to anticipate the air puff, the response correlated with the tone. The learning trials occurred daily for 5 consecutive days, followed by a 2 day rest period, and 3 consecutive days of testing. For testing using the delay paradigm, each trial was 600 ms in duration with a 500 ms tone followed by a

100 ms air puff. Under the trace paradigm, each trial was 600 ms in duration with a 100 ms tone, followed by a 400 ms “blank period” (no stimuli), then a 100 ms air puff. The eyeblink conditioning behavioral testing procedures were validated using scopolamine and d-amphetamine.

At approximately 6 months of age, approximately 12 male and 12 female F1 rabbits from each treatment group were selected for reproductive evaluation. The high dose thalidomide group included some F1 animals that came from the satellite TK group since pup mortality in this group was high. Females were injected with HCG within 3 hours prior to placement with a male. The animals were watched for approximately an hour and the female was removed to its cage once mating occurred. If the rabbits were seen fighting, a different male was chosen for mating with the female. If the animals did not mate on the first day, they were given another mating opportunity on the next day. If mating did not occur on the second day, a new male was chosen and paired with the female on a third day. If mating still did not occur, the female was listed as having no confirmed day of mating and was sacrificed and had its uterine contents examined 29 days after the last mating attempt. The F1 males chosen for reproductive evaluation were sacrificed at the end of the mating period. During a gross necropsy, their testes and epididymides were removed, weighed and fixed for microscopic evaluation. Any surviving F1 male or female rabbits that were not chosen for reproductive evaluation were sacrificed at 6 months of age and grossly necropsied. The mated F1 female rabbits were sacrificed on DG 29. They were examined for the number and distribution of corpora lutea, implantation sites, live and dead fetuses, early and late resorptions, placental abnormalities, and gross lesions. Fetuses were weighed, examined internally to determine gender, and evaluated for gross external alterations. Uteri from F1 females that did not appear pregnant were stained with ammonium sulfide to confirm a lack of implantation sites.

Sections of sciatic, tibial, fibular, and sural nerves were removed from all F1 male and female rabbits that were chosen for additional postweaning evaluation at the time of their necropsies. These tissues were preserved in neutral buffered formalin for possible future histological evaluation, but no data from such an evaluation were included in the study report.

**Results:** There was no drug-related mortality in the F0 does. No clinical signs of toxicity were observed. One doe in the control group and 2 in the 150 mg/kg group were not pregnant. One vehicle control doe was found dead on GD 27; its uterus contained 8 dead fetuses which appeared normal for their developmental age. One doe each in the 30 and 150 mg/kg thalidomide groups and 2 in the 500 mg/kg group aborted between GD 25-28 and were sacrificed afterward. The low dose doe aborted one fetus (partial cannibalization precluded evaluation), but had 9 live, superficially normal fetuses *in utero* at sacrifice. The mid dose doe aborted 3 fetuses that appeared normal for their developmental age and 3 partly resorbed fetuses (one early) with an additional conceptus unaccounted for and presumed cannibalized. One high dose doe aborted 2 partially resorbed fetuses and contained 6 more. The other high dose doe aborted one late resorption with gastroschisis and contained 9 superficially normal fetuses. Gross necropsy revealed numerous tan areas in the livers of the 2 high dose F0 does and the investigators attributed these to drug treatment, as the finding was not present in any of

the other F0 does. The investigators attributed all of the abortions to thalidomide treatment.

Maternal body weight gain of the F0 rabbits during pregnancy and lactation was not altered by thalidomide treatment. In contrast, mean food consumption was significantly ( $p \leq 0.01$ ) reduced in does from the 150 and 500 mg/kg thalidomide groups in a dose-related manner, particularly during GD 18-21 (the initiation of drug administration). During the lactation period, the high dose F0 does also consumed less food than controls.

The duration of gestation, approximately 32 days, was similar among the treatment groups. The mean number of implantation sites (9-10) per doe did not significantly differ between dose groups. Gender of the surviving pups was balanced between groups. No changes in maternal behavior were noted.

Thalidomide treatment of the F0 does was clearly fetotoxic. The percentage of does with some stillborn pups increased with dose, as did the percentage of litters where all pups died during LD 1-4 and 5-29. At 500 mg/kg, the number of stillborn pups per litter was significantly ( $p \leq 0.01$ ) greater than control and the number of liveborn pups significantly less. Pup viability was reduced significantly in both the 150 and 500 mg/kg thalidomide groups, with many but not all of the pup deaths occurring within the first 2 weeks of life. Within the first 4 days of lactation, 46.7% of the pups in the 150 mg/kg group and 65.7% in the 500 mg/kg group were found dead, moribund sacrificed, or presumed cannibalized, compared with 6.1% of controls. A significantly ( $p \leq 0.05$ ) lower percentage of dead pups in the high dose group (67.6 %) had no milk in their stomachs compared to control (80 %), demonstrating that more of the high dose pups had the opportunity and ability to nurse before their deaths. Although reduced neonatal viability in the 30 mg/kg thalidomide group did not reach statistical significance, 16.1% of the pups in this group were no longer alive by LD 4 and the reviewer believes that this is likely biologically significant, though the investigators did not. Mean litter sizes on LD 14 were  $6.6 \pm 2.1$ ,  $5.6 \pm 1.6$ ,  $4.7 \pm 1.2$ , and  $2.8 \pm 1.6$ , for the control, 30, 150, and 500 mg/kg dose groups, respectively. The reductions in litter size at 150 and 500 mg/kg were statistically significant to  $p \leq 0.05$  and  $p \leq 0.01$ .

#### Litter Viability F0 Does/F1 Pups

Thalidomide Dose	% Does w/ Any Stillborn Pups	% Does w/ All Pups Dying LD 1-4 <sup>a</sup>	% Does w/ All Pups Dying LD 5-29 <sup>a</sup>
0 mg/kg	4.3 % (1/23)	0	0
30 mg/kg	8.3 % (2/24)	4.2 % (1/24)	0
150 mg/kg	31.8 %** (7/22)	33.3 %* (7/21)	9.5 % (2/21)
500 mg/kg	43.5 %** (10/23)	56.5 %** (13/23)	13.0 % (3/23)

<sup>a</sup>Does not include litters with all stillborn pups

\*Significantly different from vehicle control group,  $p \leq 0.05$

\*\*Significantly different from vehicle control group,  $p \leq 0.01$

**Overall F1 Pup Viability**

Thalidomide Dose	Viability Index <sup>a</sup>	Lactation Index <sup>b</sup>
0 mg/kg	81.1 % (172/212)	86.8 % (145/167) <sup>c</sup>
30 mg/kg	72.2 % (148/205)	85.9 % (122/142) <sup>c</sup>
150 mg/kg	41.8 %* (69/165)	79.7 % (55/69)
500 mg/kg	18.3 %** (31/169)	71.0 % (22/31)

<sup>a</sup>Viability Index = Live pups on LD 7/Live pups on LD 1

<sup>b</sup>Lactation Index = Live pups on LD 28 (weaning)/Live pups on LD 7

<sup>c</sup>Excludes pups from 2 litters that were inadvertently mixed together and sacrificed on LD 25/28

\*Significantly different from vehicle control group,  $p \leq 0.05$

\*\*Significantly different from vehicle control group,  $p \leq 0.01$

Pups were not weighed until LD 4, and there was no difference in average pup weight between drug-treated and control animals at that time. On day 14, pups from thalidomide-treated litters were heavier than controls, though the difference was not statistically significant. On day 21, the 500 mg/kg pups were significantly ( $p \leq 0.01$ ) larger than controls (mean pup weight by litter  $338.8 \pm 53.9$  g vs.  $263.6 \pm 62.4$  g), likely due to reduced litter size with greater amounts of milk per pup available. After the lactation period, however, F1 rabbits that had come from the 500 mg/kg thalidomide-treated does did not gain as much weight as controls and were, on average, smaller than controls by 35 days of age and throughout the rest of the study. In general, feed consumption was similar among the F1 animals from the various groups.

Thalidomide was detected in the milk and plasma of all drug-treated does, including very low levels (unexplained in the report) in the milk of control does (plasma samples were not taken from control does). Thalidomide levels in milk generally exceeded those in plasma, with the exception of the high dose group during week 3 of lactation. Two animals in that dose group had unexpectedly low levels of thalidomide in their milk (approximately 1  $\mu\text{g/ml}$ ), reducing the average for that group at the 3 week time point and increasing the variability.

**Thalidomide Concentration ( $\mu\text{g/ml}$ ) in Milk and Plasma of Lactating Rabbits (Mean  $\pm$  SD)**

	Control	30 mg/kg	150 mg/kg	500 mg/kg
<b>LW* 1</b>				
<b>Plasma</b>	Not Sampled	10.18 $\pm$ 3.42	18.51 $\pm$ 11.77	21.65 $\pm$ 14.97
<b>Milk</b>	0.08 $\pm$ 0.08	13.44 $\pm$ 8.95	32.94 $\pm$ 13.17	49.12 $\pm$ 15.88
<b>LW 2</b>				
<b>Plasma</b>	Not Sampled	9.80 $\pm$ 7.50	20.56 $\pm$ 9.36	17.30 $\pm$ 10.43
<b>Milk</b>	0.06 $\pm$ 0.03	15.73 $\pm$ 1.98	35.63 $\pm$ 3.32	62.80 $\pm$ 7.81
<b>LW 3</b>				
<b>Plasma</b>	Not Sampled	3.88 $\pm$ 2.86	21.20 $\pm$ 8.46	24.61 $\pm$ 13.64
<b>Milk</b>	0.01 $\pm$ 0.01	8.18 $\pm$ 0.51	22.20 $\pm$ 3.14	15.75 $\pm$ 16.87

<b>LW 4</b>				
<b>Plasma</b>	Not Sampled	6.65 ± 1.46	14.82 ± 5.77	25.75 ± 9.37
<b>Milk</b>	0.04 ± 0.05	7.74 ± 2.90	36.08 ± 7.11	71.43 ± 30.84

\*LW, Lactation Week

The incidence of F1 pups with splayed limbs was increased in the 150 and 500 mg/kg groups compared to controls. To further investigate, microscopic evaluation was performed on several control and high dose pups (not all affected pups in either group were included) including multiple sections of the brain, spinal cord, spinal nerve roots, dorsal root ganglia, peripheral nerves, and skeletal muscle. Each of these animals was also x-rayed. The x-rays did not reveal any differences in the appearance of the skeletal systems of the affected and unaffected rabbits. There were no microscopic changes in the nervous and muscle tissues from the unaffected control animals (3/gender) or 1 male control rabbit with splayleg. Mild, focal meningitis was observed in the brain of one high dose female with splayleg, but the other nervous and muscle tissues from the animal appeared normal. Five of 6 high dose animals with splayleg had minimal nerve fiber degeneration (various locations including sciatic nerve, brachial plexus, and spinal cord) and one of these had mild neuron vacuolation of the dorsal root ganglia (slightly greater than the background level seen even in the control animals). The affected nerve fibers appeared fragmented and their morphology suggested that myelin ovoids were present. This nerve fiber degeneration may have been related to maternal thalidomide treatment, as degeneration of peripheral nerve fibers, ganglia, and posterior columns of the spinal cord is known to occur in patients taking the drug. The nerve fiber degeneration did not appear severe enough to have been a likely cause of the splayleg. Four of 6 high dose rabbits with splayleg had muscle fiber atrophy (3 mild, 1 moderate). Three of these animals also had mild muscle fiber degeneration, one had mild vacuolation as well. In the opinion of the pathologist, the changes in skeletal muscle were likely secondary to the splayleg condition and not the cause because one would expect the histopathologic changes to be more severe if they were the cause of the splayleg rather than a consequence. The investigators theorized that the increased incidence of splayleg in the pups from the mid and high dose groups was a consequence of their more rapid weight gain (too much for the developing musculoskeletal system to adequately support); since these litters were smaller, the pups had greater access to the doe's milk supply and were heavier.

Neither preputial separation nor vaginal opening was accelerated or delayed in the F1 offspring from thalidomide-treated does. Mean testicular and epididymal weights of the F1 males sacrificed at the end of the postweaning evaluation period did not differ significantly among the groups.

The delay and trace conditioning studies did not reveal any drug-related deficits in the learning ability of the control and drug-treated F1 rabbits. In the trace conditioning studies, rabbits from the 150 mg/kg group tended to learn to avoid the air puff more quickly than the control rabbits did (statistically significant,  $p < 0.05$  for females only); the investigators believed that these animals may have been more responsive to changes in their environment than controls. The high dose rabbits did not appear to have undergone undergo trace conditioning testing; the reason for this was not discussed in the study

report. Data from delay conditioning tests of the 500 mg/kg F1 rabbits were present in the study report.

Reproductive capacity was evaluated in 12 male/female pairs of F1 rabbits from the control, 30, and 150 mg/kg thalidomide groups and 11 males were mated to 13 females from the 500 mg/kg dose group. The fertility index was not statistically significantly reduced in the drug treated rabbits (90-100 % of the animals in each group mated and 83.3-100 % of the mated females were pregnant). One mated female in the 150 mg/kg group and 2 in the 500 mg/kg group were not pregnant. The investigators considered it possible that the high dose of thalidomide affected the pregnancy rate in the F1 females (of 13 paired females, one did not mate and 2 mated, but were not impregnated). This is possible, but the reviewer finds it difficult to definitively attribute these observations to drug as it is not extremely unusual for 1/13 does to not mate (after 3 attempts with 2 different males), nor for 2/12 mated animals not to be pregnant. One doe from the 30 mg/kg group and 2 from the 150 mg/kg group aborted their litters on GD 27-28. All 3 of these litters consisted of resorptions and aborted fetuses. An additional doe from the 150 mg/kg group delivered its litter prematurely (GD 29). This litter consisted of one live fetus, two dead fetuses and 3 early resorptions *in utero*, and two delivered pups (one partially cannibalized, viability at birth not clear). All of the remaining pregnant does had viable fetuses and the numbers of both early and late resorptions did not differ between the control and treated groups. All fetuses were alive and all placentae appeared normal. The percentage of male fetuses was similar among the dose groups, as were mean fetal body weights (by litter). No drug-related external abnormalities were apparent in the F2 fetuses. The numbers of corpora lutea, implantation sites and live fetuses were reduced in the F1 thalidomide-treated groups compared to controls, though the differences were not statistically significant. The investigators considered the abortions and premature delivery seen in the 30 and 150 mg/kg groups related to F0 thalidomide administration. The reviewer finds this to be a very conservative interpretation of the data, as neither abortion nor premature delivery was observed in the 10 pregnant F1 500 mg/kg does.

Thalidomide doses  $\geq 30$  mg/kg were associated with abortion in F0 does and fetotoxicity in their offspring, although no clinical signs of maternal toxicity were observed. Thalidomide was detected in the milk of F0 does. Dose-related increases in neonatal mortality were observed in litters from the thalidomide-treated does beginning with a small increase at 30 mg/kg and statistically significant larger increases at 150 and 500 mg/kg. The incidences of litters that contained stillborn pups and litters where all pups died were significantly increased at doses  $\geq 150$  mg/kg. The increased pup mortality led to smaller litters in the 150 and 500 mg/kg dose groups and the pups in these litters weighed more than controls during the lactation period, perhaps causing the increased incidence of splayleg noted in these groups. Classical eyeblink conditioning studies using the delay and trace paradigms did not reveal any learning/memory deficits in the thalidomide-exposed pups, though it is noted that the 500 mg/kg pups did not appear to have been tested using the trace paradigm. Reproductive capacity of the F1 animals from the thalidomide-treated does may have been slightly reduced, but definite attribution to the drug is difficult due to the relatively small number of rabbits tested. Of 13 females in the high dose group, one did not mate and two mated, but were not pregnant. Of 11 pregnant does each in the 30 and 150 mg/kg dose groups, there was 1 abortion, and 2

abortions and 1 premature delivery, respectively. The numbers of corpora lutea, implantation sites and live fetuses were slightly reduced in the F1 thalidomide-treated groups compared to controls, though the differences were not statistically significant.

**Oral (Stomach Tube) Fertility and General Reproduction Toxicity Study of (±) Thalidomide in Rabbits (Segment I) (Protocol 2103-002) Submitted to IND 48,177-087**

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Report dated 7/14/03, U.S. and Japanese GLP, signed QA statement present

**Animals:** New Zealand White rabbits [Hra:(NZW)SPF], 3.5-4.7 kg (males) and 3.1-4.1 kg (females) at time of study assignment, 25/sex/treatment group, plus additional untreated females to mate (1:1) to treated males.

**Diet:** \_\_\_\_\_ was offered daily. Approximately 180-185 g/day was offered to males and presumed pregnant females and approximately 150-155 g/day was offered to females prior to mating. Tap water that was further purified by reverse osmosis and containing chlorine as a bacteriostat was available *ad libitum*.

**Drug Dose and Route of Administration:** Thalidomide (Batch #574-574-00-015) was administered daily by oral gavage at doses of 10, 50, and 100 mg/kg/day (females) or 30, 150, and 500 mg/kg/day (males). Vehicle control groups received aqueous 1% carboxymethylcellulose. Doses were set based upon the results of a range finding study conducted at the laboratory. In female rabbits, doses of 150, 300 and 500 mg/kg/day appeared to inhibit ovulation and the does given  $\geq 300$  mg/kg/day had no viable litters. Male rabbits had a significant reduction in body weight gain and epididymal weights when given 500 mg/kg/day.

**Length and Conduct of Study:** Groups of female rabbits received thalidomide or vehicle for 14 days prior to mating, and continuing during the mating period (1:1 with untreated males) through GD 7. On the day of mating, each female received 20 u/kg of HCG to induce ovulation and was placed in the cage of a male rabbit. If pairs did not mate within an hour after introduction, up to 2 alternate pairings were attempted. If mating still did not occur, a second day of pairings was conducted. After mating with this first set of females, the males received thalidomide or vehicle for 14 days prior to 1:1 mating with untreated females. Treatment continued throughout the mating period until the day before sacrifice for at least 56 days of administration.

Rabbits were observed for viability at least twice daily and clinical signs were recorded before dosing and about one hour after dosing. Clinical signs were recorded once daily in the untreated does. Body weights in drug-treated does were measured daily during the period of administration, then on GDs 10, 14, 17, 20, 23, 26, and at sacrifice. Body weights of males were measured daily. Body weights of the untreated pregnant females were measured on GDs 0, 7, 10, 14, 17, 20, 23, 26, and at sacrifice. Food consumption was recorded daily for all rabbits.

Both treated and untreated female rabbits were sacrificed on GD 29. Fetuses were removed by Caesarean section and a gross necropsy was performed on the does. All gross lesions, uteri, and ovaries were saved for possible microscopic examination. The number of corpora lutea in each ovary was recorded and the numbers of implantation sites, early and late resorptions, and live and dead fetuses were determined. Uteri from rabbits that appeared not to be pregnant were stained to confirm a lack of implantation sites. Placentae were examined with regard to size, color, and shape.

Fetuses were weighed, examined for gross external abnormalities, and examined internally to determine gender.

Following at least 56 days of dosing, semen samples were collected from all male rabbits approximately one hour after thalidomide administration. These were analyzed for count and motility via CASA \_\_\_\_\_, and drug levels. On the day following the last dose of drug, males were sacrificed and underwent gross necropsy. The testes and epididymides were removed, weighed, and fixed/preserved for histological examination.

**Results:** No drug-related mortality was observed in the male rabbits. One animal each in the control, 150, and 500 mg/kg dose groups and two in the 30 mg/kg dose group died due to intubation errors.

Clinical signs of thalidomide toxicity were not observed in any of the male rabbits. Body weight gain was significantly suppressed in the drug-treated males, particularly during the first month of dosing. Total mean body weight gain was similar for the 150 and 500 mg/kg dose groups ( $0.12 \text{ kg} \pm 0.14\text{-}0.19 \text{ kg}$ ) and both were slightly less than for the 30 mg/kg group ( $0.17 \pm 0.20\text{kg}$ ), but all thalidomide groups gained significantly ( $p \leq 0.01$ ) less body weight than control ( $0.43 \pm 0.26 \text{ kg}$ ). A dose-related decrease in food consumption was also observed during the first week of treatment ( $p \leq 0.01$  compared to control).

Treatment with up to 500 mg/kg of thalidomide for 14 days did not impair the fertility of male rabbits. Neither the copulation index nor the fertility index was reduced. Dose-related increased incidences of small and/or flaccid testes were observed in the drug-treated animals. The increases in the incidence of bilateral small testes in the 150 and 500 mg/kg dose groups (4 and 7 out of 25, respectively) were statistically significant. None of the males in the control group had bilateral small testes and 2/25 in the 30 mg/kg dose group did. Both absolute testis weights and testis weight:body weight ratios were reduced in a dose-related manner in all thalidomide groups. Microscopic examination of the testes revealed a dose-related degeneration of the germinal epithelium (see table below). Multinucleate giant cells were observed in the lumen of the seminiferous tubules and, as the incidence and severity of degeneration increased, fewer round and elongating spermatids were seen lining the seminiferous tubules, though basement membranes were not exposed and Sertoli cells and spermatocytes were still present. CASA revealed no statistically significant reductions in sperm motility, count, and concentration following thalidomide treatment.

#### **Incidence and Severity of Degeneration of the Germinal Epithelium in Testes of Thalidomide-Treated Rabbits**

Severity	Vehicle	30 mg/kg	150 mg/kg	500 mg/kg
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<b>Normal Tissue</b>	6	3	1	0
<b>Minimal</b>	16	10	8	3
<b>Mild</b>	2	6	11	10
<b>Moderate</b>	0	3	4	11

Thalidomide was detected in semen. Drug levels varied greatly within dose groups, but an increase in the mean concentration of thalidomide was observed. At the 30, 150, and 500 mg/kg dose levels,  $2.67 \pm 1.23$ ,  $9.14 \pm 3.42$ , and  $15.4 \pm 5.64$   $\mu\text{g/ml}$  of thalidomide was present. It should be noted, however, that thalidomide was also detected in the semen of most control animals. The vast majority of these samples (13/22) contained very low levels of thalidomide ( $< 0.08$   $\mu\text{g/ml}$ ), but 2 of the samples contained 5.93 and 14.2  $\mu\text{g/ml}$  of the drug. No explanation was offered as to why thalidomide was present in the semen samples from vehicle-treated rabbits. For the 2 samples that contained relatively high levels of the drug, misdosing of the animals or an error in labeling the samples are both possible explanations. The reviewer notes that one sample from the 30 mg/kg group contained 0.07  $\mu\text{g/ml}$  of thalidomide and one from the 500 mg/kg group had a drug level below the quantitation limit. Both of these are in the range of most samples from the vehicle control group and the 2 high samples from the vehicle group are more in line with samples from the drug-treated rabbits. While the low levels of thalidomide detected in semen from the vehicle control group are also of concern, there did not appear to be a higher than normal background level of testicular changes in the vehicle group and neither of the 2 samples from this group that contained relatively high levels of thalidomide was associated with any significant testicular pathology. Thus, it is unlikely that these rabbits were repeatedly misdosed.

The litters sired by the thalidomide treated males all appeared normal. Litter averages for the number of implantations, litter size, early and late resorptions, and live fetuses were similar among the groups. Drug-related fetal malformations or variations were not apparent.

In the female rabbits, no drug-related mortality or clinical signs were observed. One doe each from the control and 10 mg/kg dose groups aborted their litters. The abortion in the 10 mg/kg doe was not considered related to thalidomide because none of the rabbits in the higher dose groups aborted their litters. Body weight gain was similar among all dose groups for the first 14 days of dosing (prior to cohabitation). For the first 2 weeks of gestation, body weight gain was suppressed slightly (but significantly,  $p \leq 0.01$ ) in the high dose does and more sporadically in the mid dose does. Food consumption was also slightly reduced in the high dose females during the first week of gestation. Overall, however, neither body weight gain nor food consumption differed significantly among the treatment groups when the 29 day gestation period was considered in its entirety. Thus, the biological significance of the slight changes in these parameters is questionable. Gross necropsy of the does did not reveal any drug-related changes.

Mating (84-100%) and fertility (81-96%) indices were not affected by thalidomide treatment up to 100 mg/kg, but doses as low as 10 mg/kg were associated with reduced embryonic survival. The average numbers of corpora lutea and implantation sites did not differ significantly among the treatment groups, but these values were both lower in the high dose group compared to control. Mean litter size was

reduced significantly ( $p \leq 0.01$ ) at 100 mg/kg compared to control ( $2.9 \pm 2.8$  fetuses compared to  $6.0 \pm 3.3$ ). The fetuses present at the time of caesarean section were all alive; the reduction in mean litter size in the high dose animals was due to increases in early resorptions ( $2.1 \pm 2.2$  vs.  $0.5 \pm 0.8$ ) and the percentage of does whose litters were completely resorbed (28.6 % [6/21] vs. 4.3 % [1/24]). The number of early resorptions per litter was also increased in the 10 and 50 mg/kg groups ( $1.1 \pm 1.6$  and  $1.8 \pm 2.2$ ). Although these increases were not statistically significant, the fact that the number of does with multiple early resorptions increased with dose coupled with the findings in the 100 mg/kg group suggest that they were biologically significant. Additionally, when only litters containing live fetuses are considered, there is a dose-related increase in the percentage of resorptions per litter; control,  $11.0 \pm 13.8\%$ , 10 mg/kg,  $19.3 \pm 20.3\%$ , 50 mg/kg,  $29.1 \pm 30.9\%$ , and 100 mg/kg,  $32.7 \pm 27.5\%$ . The increased percentages of resorptions were statistically significant ( $p \leq 0.05$ ) at the mid and high doses.

The placentae from all litters appeared normal, regardless of treatment group. Fetal weights were similar among treatment groups. Although the percentage of male fetuses per litter ( $67.7 \pm 27.4\%$ ) was higher than expected in the 100 mg/kg dose group, this is more likely a reflection on the smaller number of live fetuses in this group rather than a true sex-specific difference in survival. With one possible exception, external fetal alterations were observed only in 1-2 fetuses in a single litter and/or at incidences within the historical control ranges. Gastroschisis was present in 3 fetuses from 2 litters (out of a total of 15 litters) in the 100 mg/kg group. Two of these fetuses had additional abnormalities. One of them also had absent nares, snout and eyes among other malformations and the other fetus had a short snout in addition to gastroschisis. Although the incidence of gastroschisis is relatively low in the high dose litters, gastroschisis was not observed in the concurrent control animals or the historical control database provided (June 1998-June 2000) and it appears to be rare. It's possible that thalidomide treatment may be responsible for the increased incidence of gastroschisis in the rabbits, though the investigators did not believe this to be the case. It would be difficult to definitely attribute this malformation to the drug because of the reduced fetal survival at the doses that may be necessary to induce it at a high incidence. One fetus with gastroschisis from a doe given 500 mg/kg of thalidomide from GD 18-PND 28 was seen in a Segment 3 rabbit study (reviewed above). The question of whether thalidomide definitely induced gastroschisis in the rabbit fetuses is not critical because the drug is a known human teratogen, is already labeled as such, and is only available under a restricted distribution program.

Treatment of male and female rabbits with up to 500 and 100 mg/kg of thalidomide, respectively, for 14 days prior to mating did not cause impairment of fertility. Neither the copulation nor the fertility index was reduced by drug treatment. The litters sired by the male rabbits that received thalidomide with untreated female rabbits all appeared normal. However, following at least 56 days of thalidomide treatment, absolute testis weights and testis weight:body weight ratios were reduced in a dose-related manner in all thalidomide groups (30, 150, and 500 mg/kg). There was also a dose-related increase in the incidence of male rabbits with small and/or flaccid testes. Microscopic examination of the testes revealed a dose-related degeneration of the germinal epithelium. However, CASA revealed no statistically significant reductions in sperm motility, count, and concentration following thalidomide treatment. Thalidomide

was detected in the sperm of drug-treated rabbits. In the litters of drug-treated female rabbits, doses as low as 10 mg/kg were associated with reduced embryonic survival. An increased incidence of gastroschisis was observed in litters from does treated with 100 mg/kg. It may be related to drug since the historical control database suggests that it is a rare external malformation, but reduced fetal survival would make definite attribution difficult (i.e., it would not be possible to study higher doses to see if the increased incidence seen at 100 mg/kg is at the bottom of a dose response curve).

#### **OVERALL SUMMARY AND EVALUATION:**

These Segment I and III reproduction toxicity studies were submitted to fulfill part of a Phase IV commitment for NDA 20,785. The studies appear to be adequate, in general, so the pharmacologist recommends that the Division consider this part of the sponsor's Phase IV commitment fulfilled. However, the Division may want to clarify the reason for the lack of data from trace conditioning testing of the F1 rabbits from the parents that received 500 mg/kg of thalidomide in the Segment III peri/postnatal development study (Protocol 2103-001). Results from delay conditioning testing were available for this dose group and no deficits were observed.

In the Segment I study, treatment of male and female rabbits with up to 500 and 100 mg/kg of thalidomide, respectively, for 14 days prior to mating did not cause impairment of fertility. However, following at least 56 days of thalidomide treatment, testicular weights were reduced at doses  $\geq 30$  mg/kg and there was a dose-related increase in the incidence of male rabbits with small and/or flaccid testes. Microscopic examination revealed a dose-related degeneration of the germinal epithelium, although CASA revealed no statistically significant reductions in sperm motility, count, and concentration. Doses as low as 10 mg/kg given to females starting 14 days prior to mating and continuing through GD 7 were associated with reduced embryonic survival. An increased incidence of gastroschisis was observed in litters from does treated with 100 mg/kg. It may be related to drug since the historical control database suggests that it is a rare external malformation, but reduced fetal survival makes definite attribution difficult (i.e., it would probably not be possible to study higher doses to see if the increased incidence seen at 100 mg/kg is at the bottom of a dose response curve). However, an aborted fetus with the same malformation from a doe that was receiving 500 mg/kg of thalidomide was seen in the Segment III study (Protocol 2103-001). The question of whether thalidomide definitely induced gastroschisis in the rabbit fetuses is not critical because the drug is a known human teratogen, is already labeled as such, and is only available under a restricted distribution program.

When administered to pregnant/lactating does from GD 18 through PND 28, thalidomide doses  $\geq 30$  mg/kg were associated with abortion in F0 does and fetotoxicity in their offspring, although no clinical signs of maternal toxicity were observed. Thalidomide was detected in the milk of the F0 does at levels generally higher than those in plasma. Dose-related increases in neonatal mortality (birth to LD 4) were observed in litters from the thalidomide-treated does beginning with a small increase at 30 mg/kg (16.1 % vs. 6.1 % in controls) and statistically significant larger increases at 150 and 500 mg/kg (46.7 % and 65.7 %, respectively). The incidences of litters that contained stillborn pups and litters where all pups died were significantly increased at doses  $\geq 150$  mg/kg. Classical eyeblink conditioning studies using the delay and trace paradigms did

not reveal any learning/memory deficits in the thalidomide-exposed pups, though it is noted that the 500 mg/kg pups did not appear to have been tested using the trace paradigm. Reproductive capacity of the F1 animals from the thalidomide-treated does may have been slightly reduced, but definite attribution to the drug is difficult due to the relatively small number of rabbits tested. Of 13 females in the high dose group, one did not mate and two mated, but were not pregnant. Of 11 pregnant does each in the 30 and 150 mg/kg dose groups, there was 1 abortion, and 2 abortions and 1 premature delivery, respectively. The numbers of corpora lutea, implantation sites and live fetuses were reduced in the F1 thalidomide-treated groups compared to controls, though the differences were not statistically significant.

**RECOMMENDATIONS:** The sponsor should be notified that the Division considers this part of their Phase IV commitment to be fulfilled and they should include the results of these studies in the appropriate portions of the Thalomid® label. However, the Division may want to clarify the reason for the lack of data from eyeblink conditioning testing using the trace paradigm for the F1 rabbits from the parents that received 500 mg/kg of thalidomide in the Segment III peri/postnatal development study (Protocol 2103-001). It is doubtful, however, that the lack of these data will impeach the entire study and necessitate that it be repeated- thus, the recommendation that the Phase IV commitment be considered fulfilled.

Amy L. Ellis, Ph.D.  
Pharmacologist, HFD-520

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**2.6.6.7 Local tolerance**

No studies submitted.

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**2.6.6.8 Special toxicology studies**

No studies submitted.

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**2.6.7 TOXICOLOGY TABULATED SUMMARY**

None.

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**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Thalidomide has been approved for the treatment of ENL under NDA # 20-785. Nonclinical studies pertaining to that NDA have been previously submitted and reviewed by the Division of Special Pathogen and Immunologic Drug Products and the Division of Anti-Infective Drug Products. The present review contains information regarding the mechanism of action/ pharmacology only.

**Unresolved toxicology issues:** None

**Recommendations:**

The sponsor is planning to make changes to the labeling of thalidomide based on the proposed indication, multiple myeloma. After review of the new labeling and the articles cited to support the statements, we find there are not sufficient data to support all statements for the mechanism of action.

There was not adequate data to support the conclusion that thalidomide has antiproliferative activity against MM cells and that it inhibits the BMSC production of IL-6 and VEGF at relevant or therapeutically achievable exposures. Some of the effects reported for thalidomide were observed with analogs of thalidomide at much lower concentrations. In a study by Gelati et al, thalidomide had anti-angiogenic properties under the conditions of the assay, at concentrations much lower than what had been reported by other investigators. In a study sponsored by Celgene, thalidomide was shown to inhibit angiogenesis in a human umbilical artery explant model in vitro.

Suggested labeling:**Mechanism of Action**

The mechanism of action of thalidomide is not fully understood. Thalidomide possesses immunomodulatory, anti-inflammatory and anti-angiogenic properties. Available data from in vitro studies and clinical trials suggest that the immunologic effects of this compound can vary substantially under different conditions, but may be related to suppression of excessive tumor necrosis factor-alpha (TNF- $\alpha$ ) production and down-modulation of selected cell surface adhesion molecules involved in leukocyte migration. For example, administration of thalidomide has been reported to decrease circulating levels of TNF- $\alpha$  in patients with erythema nodosum leprosum (ENL), however, it has

also been shown to increase plasma TNF- $\alpha$  levels in HIV-seropositive patients. Other anti-inflammatory and immunomodulatory properties of thalidomide may include suppression of macrophage involvement in prostaglandin synthesis, and modulation of interleukin-10 and interleukin-12 production by peripheral blood mononuclear cells. Thalidomide treatment of multiple myeloma patients is accompanied by an increase in the number of circulating natural killer cells, and an increase in plasma levels of interleukin-2 and interferon-gamma (T cell-derived cytokines associated with cytotoxic activity). Thalidomide was found to inhibit angiogenesis in a human umbilical artery explant model *in vitro*. The cellular processes of angiogenesis inhibited by thalidomide may include the proliferation of endothelial cells.

Reviewer Signature \_\_\_\_\_  
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Pharmacologist

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David E. Morse, Ph.D.  
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