CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 20-785

Pharmacology Review(s)

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

NDA#: 20-785

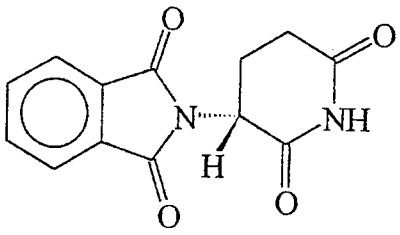
Type of Submission: BP

Reviewer: Barbara Hill

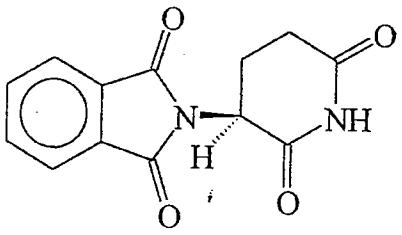
Date CDER Received: 5-11-98
Date Assigned: 5-15-98
Date Review Completed: 5-20-98
Date Accepted by Supervisor: 5-21-98

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	—
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
Stearic Acid NF	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
Anhydrous Lactose NF	[ ]	[ ]
Total Fill Weight	400.0	—

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [ ]
3) IND
4) IND
5) IND
6) NDA

]

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for

thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains the final oncogenicity protocols to be conducted in mice and rats. The final oncogenicity protocols reflect the thalidomide dose levels that were recommended by the Executive Carcinogenicity Assessment Committee received by the sponsor via FAX on 2-17-98.

DISCUSSION:

The sponsor submitted the oncogenicity protocols to the IND for thalidomide (IND 48,177; Serial #055) at the same time as to the NDA. The purpose of this review is to document that the review of the oncogenicity protocols is contained in the review of IND 48,177; Serial #055.

REGULATORY CONCLUSION:

There is no recommended regulatory action at this time from a pharmacology/toxicology perspective.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 20-785 (BP)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-105/PM/WALLING
C:WPFILES/NDAS/NDA20785/20785bp3.WPD

Concurrence Only:
HFD-105/OD/WEINTRAUB
HFD-540/TOX/AJACOBS

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

NDA#: 20-785

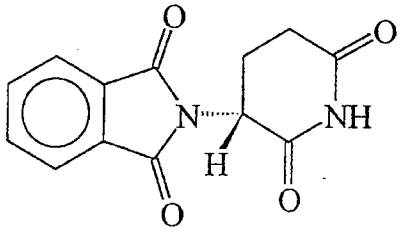
Type of Submission: BP

Reviewer: Barbara Hill

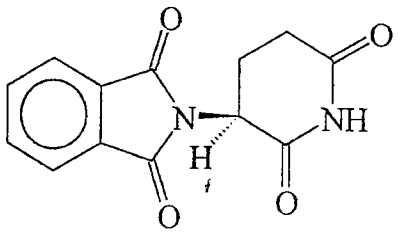
Date CDER Received: 11-4-97
Date Assigned: 11-10-97
Date Review Completed: 12-31-97
Date Accepted by Supervisor: 1-5-98

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(-)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor:
Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	–
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
Stearic Acid NF	[ ]	[ ]
[ ]		[ ]
[ ]		[ ]
Anhydrous Lactose NF	[ ]	[ ]
Total Fill Weight	400.0	-

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [ ]
3) IND
4) IND
5) IND
6) NDA

[ ]

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for

thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains the sponsor's response to toxicology concerns that were raised in an approvable letter sent to the sponsor for NDA 20-785 dated September 19, 1997.

Review of sponsor's responses to toxicology concerns raised in the approvable letter:

Note: The original request stated in the approvable letter will be provided followed by a review of the sponsor's response for each toxicology concern.

3. We request that you submit additional information concerning the historical control data for CD-1 mice and the formation of corneal crystals. In addition, it would be helpful for you to provide a potential explanation for: a) the formation of cataracts in the 14 day repeat dose toxicity study and not in the 90 day repeat dose toxicity study preformed in mice and b) the formation of corneal crystals in the 90 day repeat dose toxicity study and not in the 14 day repeat dose toxicity study in mice.

The sponsor was not able to give a reason for the finding of cataracts in two of the animals in the 14 day study. The sponsor stated that since the finding was observed only in two animals in the low dose group and in one in the high dose group in the 14 day study and in one control animal in the 90 day study suggests no relationship to treatment. The sponsor stated that the animal supplier, contract toxicology facility and bedding used were identical in both the 14 and 90 day studies. One possible explanation offered by the sponsor was that the cataracts were present pre-study but were too small to have been seen during the initial examination. In support of this explanation, the sponsor stated that cataracts were present pre-treatment in two of the animals (both males) received for study. Other pre-treatment ophthalmologic abnormalities were corneal crystals and opacity (1 female), cloudy cornea (1 female), and abnormalities of the retina and or retinal vessels (2 females). Documentation for these pre-treatment opthahalmologic observations and post-treatment effects were included with this submission. This documentation was not included in the original study report and helps to clarify the potential significance of pre-treatment effects on the eyes for interpretation of post-treatment effects. The incidences of cataract formation in the 14 day and 90 day repeat dose toxicity studies (10 mice/sex/dose group) are presented in the table below.

Dose Group	14 Day Study	90 Day Study
Control	0	1 female
30 mg/kg	--	0
50 mg/kg	2 males	--
200 mg/kg	0	--
300 mg/kg	--	0
750 mg/kg	0	--
3000 mg/kg	1 female	0

The sponsor stated that historical control data for formation of corneal crystals in CD-1 mice were not available from [ ] (the contract laboratory), the veterinarians involved in the study evaluations, a veterinary ophthalmologist [ ], or [ ] (the animal breeder). The sponsor included a copy of one journal article that examined the incidence of spontaneous corneal opacities in six strains of mice. The sponsor also included copies of five journal references that support the finding of an increased incidence of corneal crystals and an indication of corneal dystrophy with increased age in rats. The references that were included in this submission are listed below.

1) VanWinkle, TJ and Balk, MW. Spontaneous corneal opacities in laboratory mice. *Laboratory Animal Science* 36: 248-255, 1986.
2) Taradach, C., Regnier, B. And Perraud, J. Eye lesions in Sprague-Dawley rats: Type and incidence in relation to age. *Laboratory Animals* 15: 285-287, 1981.
3) Bruner, R.H., Keller, W.F., Stitzel, K.A., Sauers, L.J., Reer, P.J., Long, P.H., Bruce, R.D. and Alden, C.L. Spontaneous corneal dystrophy and generalized basement membrane changes in Fischer-344 rats. *Toxicologic Pathology* 20: Galley print provided, 1992.
4) Losco, P.E. and Troup, C.M. Corneal dystrophy in Fischer 344 rats. *Laboratory Animal Science* 38: 702-710, 1988.
5) Bellhorn, R.W., Korte, G.E. and Abrutyn, D. Spontaneous corneal degeneration in the rat. *Laboratory Animal Science* 38: 46-50, 1988.
6) Wegener, A. and Jochims, K. Clinical, histological and ultrastructural characteristics of a spontaneous corneal opacity in Sprague-Dawley rats. *Ophthalmic Research* 26: 296-303, 1994.

The first article in the list above provides evidence to suggest that mice appear to exhibit formation of corneal crystals at a lower incidence than rats, but there still appears to be an age related incidence of this finding in mice. The sponsor states that the observation that corneal crystals were observed in the 90 day and not the 14 day study is consistent with an age related effect in mice. The

sponsor also points out that corneal crystals were observed most frequently in the control group and there was no dose relationship seen for the formation of corneal crystals in the 90 day study. The sponsor believes that these observations suggest that this finding is not treatment related. The incidences of corneal crystal formation in the 14 day and 90 day repeat dose mouse studies (10 mice/sex/dose group) are provided in the table below.

Dose Group	14 Day Study*	90 Day Study
Control	0	3 females
30 mg/kg	--	1 female
50 mg/kg	0	--
200 mg/kg	0	--
300 mg/kg	--	1 female
750 mg/kg	0	--
3000 mg/kg	0	2 females

* - One female replaced pre-treatment due to corneal crystals.

Comments: Based on the additional information that the sponsor has provided (more detailed descriptions of the pre-treatment and post-treatment results of the ophthalmic examinations and the journal references), I agree that the incidence of cataract formation and corneal crystals are probably not related to thalidomide treatment in the 14 day and 90 day repeat dose toxicity studies performed in mice.

4. There were two neoplasms observed in the 13 week repeat dose toxicity study performed in CD-1 mice. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are relatively common spontaneous neoplasms in CD-1 mice, but one would anticipate that these tumors are relatively uncommon in CD-1 mice at this early a time in their life span. We request that you submit additional information on the historical control data for CD-1 mice to validate the claim that the two types of tumors observed in this study are relatively common spontaneous neoplasms in CD-1 mice. In particular, please pay special attention to clarifying at what time point in the life span of the CD-1 mice are alveolar-bronchiolar adenomas and uterine stromal polyps observed and what is their frequency level. One potential possibility for the presence of the alveolar-bronchiolar adenoma could be due to a murine virus infection of that particular animal. We request that you provide additional information on the health status of the CD-1 mice used in this 13 week repeat dose toxicity study.

The sponsor provided historical control data for CD-1 mice obtained from the Charles River 1987 publication titled "Spontaneous Lesions in the Crl:CD-1 (ICR)BR mouse". The sponsor included a copy of this publication in the submission. The bronchiolar/alveolar adenoma and uterine endometrial stromal polyp lesions were the most frequently reported findings for the lung and the uterus in the mouse in the control groups for 18 and 24 month studies. A summary of the historical control data for CD-1 mice obtained from the 1987 Charles River Publication "Spontaneous Lesions in the Crl:CD-1 (ICR)BR Mouse" is provided in the table below.

Tumor Type	Sex/Age	No. Examined	No. (%) Tumors
Bronchiolar/alveolar adenoma	Males/18 months	496	22 (4.4%)
	Females/18 months	496	14 (2.8%)
	Males/24 months	480	19 (4%)
	Females/24 months	481	14 (2.9%)
Uterine endometrial stromal polyp	Females/18 months	496	27 (5.4%)
	Females/24 months	482	16 (3.3%)

The sponsor provided information on longer term (greater than 90 days) studies with CD-1 mice which had been recently conducted at [ ] The incidences of bronchiolar/alveolar adenomas and uterine endometrial stromal polyps are summarized in the table below.

Tumor Type	Sex/Age	No. Examined	No (%) Tumors
Bronchiolar/ alveolar adenoma	Males/≥ 6 months	200	31 (16.0%)
	Females/≥ 6 months	200	12 (6.0%)
Uterine endometrial stromal polyp	Females/≥ 6 months	200	0

The sponsor stated that data from studies conducted prior to the Celgene studies produced the following (combined) incidences: alveolar/bronchiolar adenomas in 42 out of 546 males (7.7%) and in 26 out of 546 females (4.8%). No uterine stromal polyps were observed in any females. In addition, the sponsor stated that in studies that were of durations up to 90 days, no instances of either alveolar/bronchiolar adenomas or uterine stromal polyps were reported in control CD-1 mice at [ ] However, the sponsor pointed out that studies of these shorter duration typically have 10 to 15 controls/study group. Therefore, the sponsor proposes that data for these recently conducted studies are based on smaller sample populations and may have produced negative findings for this reason.

The sponsor stated that in the judgement of [ ] Manager of Pathology at [ ] there is no basis on which one can attribute either neoplasm to an untoward effect of the test article. Dr. [ ] states that the lung tumor was a single occurrence in a low-dose animal, where no other treatment-related alveolar- or bronchiolar-cell effect was observed and the uterine polyp occurred in a high dose female. However, no effect on ovarian follicular maturation or corpus luteum formation was seen, nor was there any effect on uterine endometrium. Dr. [ ] stated that small uterine polyps, as seen in this study, are viewed by some pathologists as equivocal neoplasms, perhaps related more to the function of a cut within a hyperplastic mucosa than to true neoplastic development. Dr. [ ] states that there was no test article related endometrial hyperplasia or dysplasia. Dr. [ ] concluded that both lesions were spontaneous and incidental to the test article administration since both neoplasms occurred singly, and no related non-neoplastic changes were present, and since both tumors are not unique or highly unlikely.

In this submission, the sponsor provided the results of the serology, bacteriology and parasitology testing for the mouse colony used in the 90 day repeat dose study that was supplied by [ ]. Virologic testing was performed for a sample of 10 males and 10 females upon receipt and all results were negative.

Comments: The sponsor provided evidence that the CD-1 mice used in the 90 day repeat dose toxicity study were healthy and that the alveolar-bronchiolar adenoma and uterine stromal polyp were probably not due to a murine virus infection. However, the cause of these two findings is still unclear. The sponsor provided 10 year old historial data that these two types of lesions are a relatively common spontaneous occurrence in longer term studies (i.e. 18 and 24 month studies). The appropriateness of this data for the current study is questionable. In addition, the sponsor provided evidence that the alveolar-bronchiolar adenoma lesion was a relatively common spontaneous lesion in 6 month studies in CD-1 mice. However, the evidence that the sponsor provided also showed that the uterine stromal polyp was not a spontaneous lesion finding in studies of 6 month or less duration and that the alveolar-bronchiolar adenoma lesion was not a spontaneous lesion finding in studies of 90 days or less. Therefore, the sponsor relied on the opinion of Dr. [ ] Manager of Pathology at [ ] to determine that these two lesions were probably not related to the test article. I still believe that there is the possibility that the two observed lesions seen in the 90 day repeat dose toxicity study in mice may be related to test article administration. The conduct of the 2 year carcinogenicity study in CD-1 mice (the protocol for this study is included in the current submission) will help to determine if this is a treatment related finding or not.

5. We are concerned about the AUC values obtained in the 52 week repeat dose toxicity study in dogs. The maximum AUC obtained in this study (approx. 100 µg•hr/ml) is substantially lower (approx. ¼X) than was obtained in the 7 day repeat dose pharmacokinetics study (approx. 400 µg•hr/ml) performed in dogs. We request that you submit any additional information that you may have to provide an explanation for this observation. In particular, information concerning the status of the dogs fed or fasted state prior to dose administration would be quite helpful. In dogs, the pH in the stomach varies according to how recently the dogs have eaten and this could have a dramatic effect on the rate of spontaneous hydrolysis of thalidomide.

The sponsor has submitted the pharmacokinetic results from Day 1, 178 and 364 for the 52 week dog study. In the original submission the sponsor had only submitted the Day 1 results from the one year dog study. The sponsor anticipates that the final report for this study will be completed in the final quarter of 1997. The sponsor highlighted the difference in the feeding regimen used for the rodent and dog studies. The rodents received the test article via gavage in a 1% carboxy methyl cellulose suspension and the dogs received the test article in gelatin capsules. The sponsor stated that all dogs were fed 1 hour after dosing in the 52 week study and that feeding was restricted in the 7 day study but that the timing relative to dosing was not provided in this study. The sponsor emphasized that absorption was found to be prolonged and saturated as the dose was increased to the 1000 mg/kg/day dose. There was also evidence that absorption was incomplete in that white matter was observed in the feces of many dogs. The sponsor stated that it is reasonable to assume that this white matter was unabsorbed drug.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1, 178 and 364 in Beagle dogs are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml) Mean ± SD			T_{max} (hr) Mean ± SD			$T_{½}$,e (hr) Mean ± SD			$AUC_{0\text{-}24hr}$ (µg•hr/ml) Mean ± SD		
		Day 1	Day 178	Day 364	Day 1	Day 178	Day 364	Day 1	Day 178	Day 364	Day 1	Day 178	Day 364
43*	M	2.0± 0.7	2.9± 0.5	2.7± 0.7	2.8± 2.6	2.0± 0.0	1.8± 0.5	2.7± 0.3	2.0± 0.5	2.1± 0.6	14.7± 12.1	16.9± 3.1	18.5± 13.7
43*	F	2.4± 1.7	3.7± 1.5	2.1± 0.5	1.8± 1.1	2.0± 0.0	0.8± 0.5	3.6± 3.5	2.0± 1.3	9.6± 11.6	7.8± 5.8	17.2± 6.4	16.1± 13.4
200*	M	2.6± 0.6	4.3± 1.5	3.5± 0.6	3.0± 1.1	2.7± 1.0	2.0± 0.0	3.9± 2.5	7.5± 6.4	4.4± 2.7	26.6± 16.3	37.5± 9.6	24.7± 16.0
200*	F	5.0± 2.7	7.1± 3.1	4.4± 0.8	5.5± 9.1	3.0± 1.7	2.5± 1.0	2.0± 0.9	2.5± 0.9	7.2± 11.0	49.2± 49.0	64.8± 41.4	41.3± 34.3
1000#	M	7.9± 3.9	15.2± 1.9	9.5± 1.8	6.8± 7.4	4.8± 2.1	2.7± 1.6	21.7± 23.6	5.2± 3.8	16.5± 35.1	104.5± 61.0	179.1± 57.0	82.3± 39.4
1000#	F	8.6± 3.2	15.4± 4.6	9.0± 1.9	11.8± 10.2	3.3± 1.5	2.5± 1.2	7.3± 5.1	7.6± 7.5	30.9± 46.6	112.1± 74.5	146.8± 55.10	97.8± 37.5

* - n = 6 dogs for day 1 and 178 values and 4 dogs for day 364 values.
# - n = 8 dogs for day 1 and 178 values and 6 dogs for day 364 values.

The sponsor pointed out that the inter-animal variability in pharmacokinetic parameters was quite large in the 52 week dog study. The AUC coefficients of variation were greater than 100% and coincident wide, overlapping ranges of AUCs observed across dose levels. The sponsor emphasized that there were 4 to 8 dogs/sex/dose in the 52 week study but that the 7 day dog study had only 1 dog/sex/dose. The sponsor compared the AUC values for the 1000 mg/kg/day dose

for both the 7 day and 52 week study since this dose was common to both studies. The values from the 7 day dog study for male and females on Day 1 were 450 and 350 µg•hr/ml, respectively, and on Day 7 were 393 and 205 µg•hr/ml, respectively. The values from the 52 week dog study for male and females on Day 1 were 104.5±61.0 and 112.1±74.5 µg•hr/ml, respectively, on Day 187 were 179.1±57.0 and 146.8±55.10 µg•hr/ml, respectively, and on Day 364 were 82.3±39.4 and 97.8±37.5 µg•hr/ml, respectively. The sponsor's argument is that while the 7 day values are higher, given the variability observed, these single observations are considered to not differ substantially from those observed in the 52 week study. The sponsor also re-emphasized the point that unabsorbed white substance was noted in the feces of dogs in the 7 day and 52 week studies.

Comments: Based on the pharmacokinetic results that the sponsor submitted for the 52 week dog study, I accept the possibility that the difference in AUC values for the 7 day and 52 week dog studies may not be that significant due to the variability of absorption of thalidomide in the gelatin capsules. Since the 7 day study only obtained pharmacokinetic samples from 1 animal/sex/dose group, there does exist the possibility that these values were on the high end of the spectrum. This result does lend support to the fact that it is crucial to obtain toxicokinetic data in the toxicology animal studies that are conducted with thalidomide due to this large variability in absorption of thalidomide which is probably related to the inherent low water solubility of the test substance.

6. We are concerned about the stability of thalidomide (due to spontaneous hydrolysis) under the assay conditions for the *in vitro* genetic toxicology studies that investigated the potential for thalidomide to induce mutations. It is unclear as to the stability of thalidomide in the media used to conduct the two *in vitro* genetic toxicology studies. We request that you submit information about the stability of thalidomide under the conditions of the two *in vitro* genetic toxicology studies conducted for thalidomide. We recommend that you make the following modifications to the two carcinogenicity (rat and mouse) protocols: a) delete the clinical pathology assessment at week 104 due to these results may be confounded by age related toxicities, b) draw blood from a satellite group of mice (not the animals to used for the main study) for the week 54 clinical pathology assessment, and c) conduct histopathological examination of all of the tissues from all of the dose groups in both carcinogenicity assays.

The sponsor stated that the thalidomide was dissolved in DMSO to generate a stock solution and then diluted in additional DMSO prior to use in the *in vitro* genetic assays. The sponsor states that the stability of thalidomide in this solvent was shown to be at 80% of nominal after dosing of the relevant cultures. The liquid cultures and molten agar used in the Ames test were buffered at pH 7.4. The sponsor stated that the half life of thalidomide under these conditions is ≥2.5 hours. The sponsor believed that there would likely be a substantial but unquantifiable proportion of the thalidomide test article present during the sensitive period of bacterial growth. The sponsor said that the stability of thalidomide in solid agar is unknown.

The sponsor stated that incubation of the test article thalidomide with the dividing cells in the second *in vitro* genetic assay, the AS52/XPRT assay, was for a period of 5 hours at 37°C at

pH 7.4 in buffered fetal calf serum. The half life of thalidomide in human serum is ≥1.5 hours under these conditions depending on the protein concentration. Assuming the half life is similar in fetal calf serum, the sponsor proposed that ≥10% of the thalidomide present at the initiation of the incubation would be present at the end. The sponsor estimated that the average exposure of the drug during the 5 hour incubation would therefore be expected to be in the range of 45% of the initial concentration.

The sponsor included the revised protocols for the rat and mouse carcinogenicity protocols in this submission. The sponsor stated that the modifications requested have been made to both studies. The review of these two carcinogenicity protocols will be conducted later in this document.

Comments: The information provided by the sponsor to address the concern of the instability of thalidomide under the conditions of the *in vitro* genetic toxicology studies does not alleviate this concern. If anything, the information provided provides additional support for the concern. ✓ However, it is understood by this reviewer that it would be difficult to address the question in any other way than what was supplied by the sponsor. The negative result in the *in vivo* genetic toxicology study conducted by the sponsor provides some evidence that thalidomide is not mutagenic. In addition, the willingness of the sponsor to conduct phase IV carcinogenicity studies in rat and mouse will provide data to determine the potential carcinogenic risk posed by the long ✓ term use of thalidomide.

7. We recommend that you resubmit the two carcinogenicity (rat and mouse) protocols with the results of their respective 90 day dose range studies to support the dose selection for these studies. Please be advised that the two carcinogenicity protocols, along with their respective 90 day dose range studies, will be submitted to the executive Carcinogenicity Assessment Committee (CAC) for evaluation and recommendations. The recommendations from the executive CAC evaluation of the two carcinogenicity protocols will be shared with you.

The sponsor submitted the revised carcinogenicity rat and mouse protocols, the respective 90 day dose range studies and the rationale for the dose selection in the carcinogenicity protocols.

Comments: The review of this information will be conducted later in this document.

8. We recommend that you include full hematological and clinical chemistry profile measurements in both of the reproductive toxicity dose range finding studies in male and female rabbits at appropriate time points in this study (i.e., day 7 and study termination). We recommend that you evaluate mating performance in the reproductive toxicity dose range finding study in male rabbits.

9. We recommend that you evaluate mating performance in the Segment I reproductive toxicity study in rabbits.

10. We recommend that you evaluate a measure of sexual maturation in the Segment III reproductive toxicity study in rabbits. It is also recommended that you evaluate some parameters of development in this study (i.e., measurements of learning capacity, physical strength, and motor coordination).

11. We recommend that you resubmit the Segment I and III reproductive toxicity protocols after completion of the reproductive toxicity dose range finding studies to support the dose selection for these studies.

The sponsor stated that the reproductive toxicity protocols included in the submission of NDA 20-785 were drafted by [ ] and that the above comments have been forwarded to [ ] and are under consideration. The sponsor stated that responses and revised protocols will be submitted within 2 to 3 weeks.

Comments: A review of the responses to question 8 - 11 above and the revised reproductive toxicity protocols will be performed when they are submitted to the Agency by the sponsor.

Review of mouse and rat carcinogenicity protocols, corresponding dose range finding studies and dose selection rationale:

1) 104 week mouse oncogenicity study

a) Summary of the results for the 13 week oral toxicity study of thalidomide in mice (Study number N002185B)

The study was conducted at [ ] in compliance with GLP regulations. CD-1 mice ([ ]) were administered a 1% carboxymethyl cellulose (CMC) aqueous suspension of thalidomide daily by gavage, in a dose volume of 10 ml/kg for 13 weeks at doses of 30, 300 and 3000 mg/kg. Control animals were administered vehicle alone. This study was designed to evaluate toxicity and to obtain plasma samples for the calculation of pharmacokinetic parameters. The main toxicity groups consisted of 10 mice/sex/dose, the interim clinical pathology groups (urine and blood samples collected on day 38) consisted of 5 mice/sex/dose, and the satellite pharmacokinetic groups consisted of 3 mice/sex/time point for collection of plasma samples at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours post dose administration on Days 1 and 91.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, clinical pathology (hematology and serum chemistry), urinalysis, organ weights (adrenals, brain with brainstem, kidneys, liver, thymus, epididymides and gonads {ovaries and testes}) and gross necropsy. Representative samples from all gross lesions and from a select group of organs and tissues [adrenals, aorta, bone marrow (femur), brain with brain stem (medulla/pons, cerebellar cortex and cerebral cortex), cecum, colon, duodenum, esophagus, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph node, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, spleen, stomach, testes (with

epididymides), thymus, thyroids (including parathyroids), trachea, urinary bladder, and uterus] were preserved for histopathological evaluation. The following tissues were preserved for possible future examination if indicated by signs of toxicity or target organ involvement [cervical spinal cord, exorbital lacrimal glands, eyes (both with optic nerve), femur (including articular surface), lumbar spinal cord, mammary gland (female), salivary glands (mandibular), seminal vesicles, skin, mid-thoracic spinal cord, muscle (thigh), vagina and cervix].

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on body weight, food consumption or serum chemistries were noted in this study. The ophthalmologic effects noted in this study, formation of corneal crystals, was probably not related to treatment. The rationale for this decision is that there was no dose response effect elicited for this finding (3 females in control group, 1 female in the low dose group, 1 female in the mid dose group, and 2 females in the high dose group) and literature references submitted by the sponsor suggest that this may be an age related effect. The consistent clinical observation noted in this study was the formation of discolored urine. These notations of discolored urine were documented for the mice in the satellite group during obtaining blood for plasma samples. Discolored urine occurred across all treated dose groups in the males and in the mid- and high-dose groups in the females with no such observations in the control group. There was a definite increase in the number of notations of discolored urine as the dose level increased which suggested a dose related treatment effect. A non-GLP study was conducted and showed that the discoloration was not due to occult blood. A potential explanation for the urine discoloration may be the transient appearance of chromogenic byproducts of thalidomide in the urine. There is evidence in the literature to support this possibility.

A dose dependent decreasing trend was seen in lymphocyte counts. This did not obtain statistical significance from the day 38 measurement but obtained statistical significance for the 300 mg/kg dose in male mice and the 3000 mg/kg dose in female mice from the day 92 measurement. These data may suggest a treatment induced decrease in circulating lymphocyte numbers after chronic treatment in mice. There was a significant increase in the liver weights in the 3000 mg/kg dose group for both male and female mice. There was also a significantly higher organ-to-weight ratio for livers in the 300- and 30000-mg/kg dose groups for both sexes. This increase in liver weight correlated with the histopathologic change noted (centrilobular hepatocellular hypertrophy) after administration of thalidomide in these CD-1 mice. This lesion was observed in 10/10 male mice in all three dose groups. The severity of this change in the male mice showed a dose related increase. It was minimal for the low dose males, mild for the mid dose males and moderate for the high dose males. Mild centrilobular hepatocellular hypertrophy was observed in 10/10 high dose female mice. Centrilobular hepatocellular hypertrophy was not observed in the low and mid dose female mice. There were two neoplasms observed in this study. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. The contract lab concluded that since these were isolated lesions and since these are relatively common spontaneous neoplasms in CD-1 mice, both tumors were incidental and not related to the administration of the test article. I do not concur with this assessment. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are relatively common spontaneous neoplasms in CD-1 mice, but I

would anticipate that these tumors are relatively uncommon in CD-1 mice at this early of a time in their life span. It will be important to examine the results of the 2 year carcinogenicity study in mice closely to determine if these findings are significant or not.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 90 in CD-1 mice are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (μg/ml)		T_{max} (hr)		$T_{½}$,e (hr)		$AUC_{0\text{-}24hr}$ (μg*hr/ml)	
		Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
30	M	1.86	2.56	0.50	0.60	0.40	27.23	2.89	12.59
30	F	3.78	3.97	0.50	0.23	0.86	68.16	7.23	16.00
300	M	11.30	14.18	1.29	0.90	2.18	3.43	65.56	91.61
300	F	21.62	10.36	1.87	1.41	1.37	2.73	132.18	82.81
3000	M	31.26	27.72	1.00	1.50	ND	2.64	552.09	252.35
3000	F	46.11	16.00	3.00	1.91	2.55	2.48	647.66	172.81

ND - Not determined because not able to fit to curve due to unusual or insufficient data.

Following single administration, C_{max}, $t_{½e}$ and AUC increased with dose. When C_{max} is compared versus dose there is a significant blunting of the C_{max} at the 3000 mg/kg dose relative to the lower doses for both sexes. This lack of dose proportionality suggests a saturation of absorption at the high dose. The increase in AUC was more proportional when the low and mid doses were compared but less than proportional when the high dose is compared to the mid dose. These data are consistent with saturation of absorption and continuation of absorption processes for a longer period of time at the higher dose relative to the lower doses. There was no significant differences between males and females.

Following 90 days of repeated daily oral administration, C_{max}, $t_{½e}$ and AUC increased with dose. Measurable thalidomide (0.29 to 0.97 μg/ml) was detected in the plasma at the pre dose time period for females at all doses and for males at the highest dose. Consistent with the observations following single administration, when the C_{max} was compared to dose there was a blunting of the C_{max}'s at the 3000 mg/kg dose, relative to the low and mid dose. There was also an increase in T_{max} with increased dose. These data suggest that a saturation of absorption at the high dose during the earliest absorption phase. The increase in AUC with does is less than proportional at the high dose when compared to the low dose. The sponsor showed that when the AUC versus dose is plotted, the slope of the regression lines for Day 90 is significantly lower compared to Day 1 data. This further deviation from dose proportionality at higher doses with repeated dosing suggests a more pronounced effect on saturation of oral absorption. These data

suggest that absorption of thalidomide continued for a longer period of time at higher doses relative to lower doses and that this effect was more pronounced following repeat dosing for 90 days as compared to single dose administration. The persistence of low levels of thalidomide and the shape of the AUC versus dose curves also suggest other processes may also be saturated with repeated administration.

b) Review of the rationale for dose selection for the 104 week mouse oncogenicity study

The sponsor selected doses for the mouse oncogenicity study based on the results of the 13 week repeat dose toxicity study in mice described above. The sponsor stated that the selection criteria were based on the criteria described in the International Conference on Harmonization (ICH) document "Dose Selection for Carcinogenicity Studies of Pharmaceuticals". The ICH document describes six main categories of criteria for selecting the high dose for carcinogenicity studies: 1) maximum tolerated dose (MTD), 2) area under the plasma concentration-time curve (AUC)-ratios, 3) dose limiting pharmacodynamic effects, 4) saturation of absorption, 5) maximum feasible dose, and 6) limit dose.

The high dose for the mouse carcinogenicity study is proposed to be 2000 mg/kg, given as a single daily oral administration by gavage. The sponsor states that rationale for choosing this dose was based on the pharmacokinetic data generated as part of the 13 week mouse study. These data demonstrated that absorption of orally administered thalidomide in mice appears to become increasingly saturated at about 1553 mg/kg for females and 2183 mg/kg for males according to the sponsor's estimation. At higher oral doses the saturation of absorption increased such that the AUC produced by 3000 mg/kg averaged only 15% of that expected when compared to the AUC of the 30 mg/kg dose. Thus, the sponsor believes that the application of the ICH criteria for saturation of absorption appears to be the most reasonable criteria for selecting the high dose for the mouse carcinogenicity study.

The low dose for the mouse carcinogenicity study is proposed to be 100 mg/kg. This dose is projected to produce approximately the same AUC in mice as the AUC produced in humans by the maximum proposed clinical dose of 400 mg. (Note: The AUC is ~36.4 ± 9.55 μg*hr/ml after a clinical dose 400 mg/day.)

The middle dose is proposed to be 1050 mg/kg, which is the median dose between the low and high dose.

Comments: I recommended doses of 100, 1000 and 2000 mg/kg/day for the mouse carcinogenicity study to the Executive CAC members based on the information supplied by the sponsor. The mouse carcinogenicity protocol and corresponding recommendation for dose selection were presented to the Executive CAC members (Ron Steigerwalt, Ph.D., HFD-510, Acting Chair; Paul Andrews, Ph.D., HFD-150, Alternate Member; C. Joseph Sun, Ph.D., HFD-570, Alternate Member) on December 9, 1997. Discussion during this meeting focused on: 1) The measurements for pharmacokinetic determination were made on the parent drug only with no measurements of any metabolite levels, and 2) The pharmacokinetic data demonstrate that exposure with increased

dose is non-linear but did not determine the dose at which saturation of absorption occurred for thalidomide. Therefore, the executive committee members recommended, with my concurrence, using doses of 0, 100, 1000 and 3000 mg/kg/day for both male and female mice. The high dose level was based on the maximum feasible dose determined in the 13 week toxicity study conducted in mice.

c) Review of protocol for the 104 week oncogenicity study in mice

Title: 104 week Oncogenicity Study in Mice
Protocol number: Not stated
Performing organization: [redacted]
Species/Strain: CD-1(ICR)BR mice (4 weeks old at initiation of dosing)
Number of animals: 80/sex/dose
Duration: 104 weeks
Route: Oral (gavage) at 10 ml/kg/day
Dose Levels: 0 (control; vehicle), 100 mg/kg/day, 1050 mg/kg/day and 2000 mg/kg/day. The vehicle will be 1% carboxymethyl cellulose aqueous solution.
Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14; once every 4 weeks thereafter; at week 104; and at termination.
5) *Food consumption* - 1X/week for weeks 1-14; and every two weeks thereafter.
6) *Ophthalmology* - performed prior to initiation and at weeks 52 and 104. Both macroscopic and ophthalmoscopic exams of the anterior portion, optic media and ocular fundus will be conducted.
7) *Clinical pathology* - performed at week 52. There will be 10 mice/sex/group removed for terminal bleeding and discarded without necropsy. The measurements will consist of total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count, differential leukocyte count, mean corpuscular hemoglobin volume (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).
8) *Unscheduled sacrifices and deaths* - Gross necropsies will be conducted on all moribund animals and on all animals not surviving to termination.
9) *Gross necropsy* - performed at study termination which includes examination of the external surface of the body, all orifices (including the nasal and paranasal sinuses), the cranial, thoracic, and abdominal cavities and their contents.

10) *Organ weights* - performed at study termination and the following tissues will be weighed wet: adrenals, brain, heart, kidney, liver, ovaries, testes, and thyroid/parathyroid (weighed post-fixation).

11) *Histopathological examination* - The following tissues from each necropsied animal will be preserved in 10% neutral buffered formalin: adrenals, aorta, bone with bone marrow (sternum), bone marrow smear, brain (3 levels-fore, mid and hind), eye including optic nerve (2), gallbladder, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), gonads (ovary {2} and testis with epididymides {2}), gross lesions, harderian gland, heart, kidney (2), larynx, liver (all lobes examined), lung with bronchi (2), lymph nodes (mandibular, mesenteric, mediastinal and regional when applicable), mammary gland (female only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular/sublingual, submaxillary), sciatic nerve, skeletal muscle (quadriceps), skin, spinal cord (cervical, thoracic, lumbar), spleen, thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus (both horns and cervix, and vagina . All of the tissues from all dose groups and animals found dead or sacrificed as moribund will be examined microscopically.

12) *Statistical evaluation (as deemed appropriate)* - performed for body weights, food consumption, hematology, organ weights and organ/body and brain weight ratios, incidence of histopathologically proven tumors and survival data.

Comments: The sponsor submitted the first version of the mouse oncogenicity study with the NDA submission. The sponsor has incorporated all of the recommendations made for the first version with this second protocol. Therefore, I have no additional recommendations at this point except for the dose range for this study that was described in the previous section.

1) 104 week rat oncogenicity study

a) Summary of the results for the 13 week oral toxicity study of thalidomide in rats with neurobehavioral assessment (Study number N002124A)

This study was conducted at [ ] in compliance with GLP regulations. Fischer rats [CDF(F-344/[ ] were administered a 1% CMC aqueous suspension of thalidomide daily by gavage. Ten rats/sex/dose received daily doses of vehicle control and 30, 300 and 3000 mg/kg in a dose volume of 10 ml/kg for 91 consecutive days (main study animals). Satellite groups (10/sex/dose) were used for pharmacokinetic sampling and thyroid function testing. Plasma samples were collected (0, 2, 8 and 18 hrs) post dose administration on Days 1 and 90. Plasma samples were obtained from 5 rats/sex/dose at 0 and 8 hours after Day 1 and Day 90 and from 5 rats/sex/dose at 2 and 18 hours after Day 1 and Day 90.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, neurobehavioral assessment including the functional observation battery (FOB) and motor activity, clinical pathology

(hematology and serum chemistry; coagulation parameter including prothrombin time and activated partial thromboplastin time were measured at termination), thyroid function parameters, and urinalysis. Two types of necropsies were performed in this study. The tissues of the first 6 rats/sex/dose were fixed by whole body perfusion to facilitate histological evaluation of potential neuropathology, whereas tissues of the remaining 4 rats/sex/dose were preserved by traditional (immersion) measures for histopathological evaluation. Organ weights (liver, kidneys, adrenals, thymus and gonads) were collected on those animals that were fixed by immersion (4 rats/sex/dose). Representative samples from all gross lesions and from a select group of organs and tissues [adrenals, aorta, bone marrow (femur), brain with brain stem (medulla/pons, cerebellar cortex and cerebral cortex), cecum, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lungs, lumbar spinal cord, mammary gland (female), mesenteric lymph node, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, spleen, stomach, sural nerve, testes (with epididymides), thymus, thyroids (including parathyroids), trachea, urinary bladder, and uterus] were preserved for histopathological evaluation. The following tissues were preserved and possible future examination will be performed if indicated by signs of toxicity or target organ involvement [cervical spinal cord, exorbital lacrimal glands, eyes (both with optic nerve), femur (including articular surface), salivary glands (mandibular), seminal vesicles, skin, mid-thoracic spinal cord, muscle (thigh), vagina and cervix].

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on clinical observations, ophthalmic measurements or urinary parameters were observed in this study. There were thalidomide effects on body weight throughout the study at all dose levels and in both sexes. Low group mean body weights relative to vehicle control were evident within as few as eight days of thalidomide treatment and persisted to some degree until termination at Day 91. The dose response effect of thalidomide on body weight was more consistent for male rats than for female rats. Male rats showed the following percent body weight decreases after 90 days of treatment: 8.2% (30 mg/kg), 11.3% (300 mg/kg) and 18.5% (3000 mg/kg). Female rats showed the following percent body weight decreases after 90 days of treatment: 6.0% (30 mg/kg), 7.0% (300 mg/kg) and 5.6% (3000 mg/kg). In addition, even though body weight was significantly reduced for female rats in all dose groups, the degree of effect on body weight was not as great in female rats as was observed in male rats. Male rats had a decreased food consumption in all dose groups but female rats showed no treatment related effect in food consumption.

The functional observational battery that was conducted to assess potential neurobehavioral effects revealed a decrease in forelimb grip strength in the 3000 mg/kg/day males at weeks 4, 8 and 13, which was the group with the greatest deficiency in body weight gain at those time points. The sponsor notes that the decreased body weight gain may have contributed to the decreased grip strength. Six of ten males in the 3000 mg/kg/day dose group exhibited drooping eyelids (ptosis) or eyelids that were completely shut at week 13.

Treatment related effects were seen in hematology parameters. The most noticeable effect was a dose dependent decrease in platelets seen in all dose groups at both the 6 and 13 week timepoints. This effect was more pronounced in male animals and ~~their~~ there was ~25% decrease in the high dose animals at both timepoints. The contract lab stated that the decreased platelet values fell within historical values and are of questionable toxicological significance. However, I believe that

since this decrease was statistically significant, showed a dose dependent response and was seen in the 14 day toxicity study described above, it is a significant effect. In male rats, decreased red blood cells and increased mean corpuscular volume and mean corpuscular hemoglobin might be indicative of mild anemia. No treatment related effects on coagulation values were observed in this study. Indications of minor leukopenia including lymphocytes, neutrophils, monocytes and eosinophils in treated males were also present. The treatment related significance of this may be questionable since there was no clear dose response observed and this effect was not seen consistently at both timepoint measurements.

A treatment related effect on thyroid measurement parameters was observed in this study. A significant decrease in T3 levels was seen in all the female animal dose groups and in the mid dose male animal dose group. The effects on T3 levels were not dose dependent but showed an ~28% decrease in all female animal dose groups and an ~25% decrease in the mid dose male animal group. A dose dependent treatment related effect was seen in both total and free T4 levels in both male and female animals. The effect was more pronounced in female animals with the first significant decrease being observed in the low dose group for both the total and free T4 levels (~53% decrease in total T4 and ~54% decrease in free T4 levels in the high dose group). In male animals, significant decreases were observed in the mid and high dose groups (~37% decrease in total T4 and ~44% decrease in free T4 levels in the high dose group).

Thymus weight decreased in all treated groups relative to the corresponding vehicle control levels. A possible dose response relationship was more consistent in the male rats than in female rats. Thymus weights were statistically significantly decreased relative to vehicle control in both the male and female animals in the mid dose group and in the male animals in the high dose group. No other treatment related effects on organ weights was observed in this study. No treatment related histopathological effects were seen in this study.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 90 in Fischer 344 rats are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml)		T_{max} (hr)		$T_{½,e}$ (hr)		$AUC_{0\text{-}t8hr}$ (µg*hr/ml)	
		Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
30	M	7.47	7.68	2.36	2.00	1.60	4.06	45.35	57.82
30	F	10.40	10.78	2.28	3.03	1.84	2.75	63.99	91.04
300	M	21.80	14.94	3.81	3.84	4.61	11.49	289.34	197.92
300	F	31.07	20.56	3.72	3.49	11.75	9.86	347.22	280.62
3000	M	40.19	19.84	5.25	1.14	19.00	43.78	537.10	322.52
3000	F	41.53	36.00	18.00	3.96	ND	8.75	681.66	483.34

ND - Not Determined, not able to determine from the data.

Pharmacokinetic parameters from the 90 day repeat dose study in rats demonstrated a similar profile to that seen in the 90 day repeat dose study in mice described above. The plasma thalidomide concentrations increased with dose in a non-linear manner showing a blunting of C_{max} and a plateau of AUC with increased dose. This is consistent with pharmacokinetic data obtained in the mouse study which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. There was little evidence of accumulation which is consistent with the Day 90 AUC values not being significantly greater than the Day 1 AUC values.

b) Review of the rationale for dose selection for the 104 week rat oncogenicity study

The sponsor selected doses for the rat oncogenicity study based on the results of the 13 week repeat dose toxicity study in rats described above. The sponsor stated that the selection criteria were based on the criteria described in the International Conference on Harmonization (ICH) document "Dose Selection for Carcinogenicity Studies of Pharmaceuticals". The ICH document describes six main categories of criteria for selecting the high dose for carcinogenicity studies: 1) maximum tolerated dose (MTD), 2) area under the plasma concentration-time curve (AUC)-ratios, 3) dose limiting pharmacodynamic effects, 4) saturation of absorption, 5) maximum feasible dose, and 6) limit dose.

The high dose for the rat carcinogenicity study is proposed to be 300 mg/kg given as a single daily oral administration by gavage. This choice was made by the sponsor on the basis of a decrease in weight gain for males and females which averages roughly 10% at this dose level in the 13 week study. The sponsor states that the decreased weight in the 300 mg/kg dose group appears to fit the MTD criteria defined in the ICH document. The sponsor believes that this is the best match to any of the six categories of criteria.

The low dose for the rat carcinogenicity study is proposed to be 20 mg/kg. This dose is projected to produce approximately the same AUC in rats as that produced by the maximum proposed human dose of 400 mg. (Note: The AUC is ~36.4 ± 9.55 µg*hr/ml after a clinical dose 400 mg/day.)

The middle dose is proposed to be 160 mg/kg, which is the median dose between the low and high dose.

Comments: It appears that male rats are more sensitive to thalidomide administration than mice based on the dose dependent decrease in body weight which was seen in male rats but not mice. The sponsor has proposed that the decreased weight seen in the 13 week repeat dose study is the criteria for selection of the MTD for males and females for the carcinogenicity study. I concurred with this rationale for the male rats because the decrease in body weight exhibited a dose dependent trend [8.2% (30 mg/kg), 11.3% (300 mg/kg) and 18.5% (3000 mg/kg)] and obtained the 10% level at the 300 mg/kg dose. However, the same trend in body weight was not seen for female rats [6.0% (30 mg/kg), 7.0% (300 mg/kg) and 5.6% (3000 mg/kg)]. There was no dose at which a 10% decrement in body weight was achieved for female rats in this study. However, the pharmacokinetic data for the parent compound (refer to previous section) suggest that there may be a saturation of absorption

in the female rats at the 300 mg/kg dose. With a 10 fold increase in dose (300 mg/kg to 3000 mg/kg) there is less than a 2 fold increase in AUC values. Therefore, I recommended doses of 20, 150 and 300 mg/kg for the rat carcinogenicity study to the Executive CAC members. The proposed basis for the dose selection in male rats was the MTD and in female rats was saturation of absorption.

The rat carcinogenicity protocol and corresponding recommendation for dose selection were presented to the Executive CAC members (Ron Steigerwalt, Ph.D., HFD-510, Acting Chair; Paul Andrews, Ph.D., HFD-150, Alternate Member; C. Joseph Sun, Ph.D., HFD-570, Alternate Member) on December 9, 1997. After extensive discussion of the dose selection for the rat carcinogenicity study, the committee members decided that they needed additional information on body weight decreases and body weight gain decreases to determine a proper dose selection for the rat carcinogenicity study. There was some concern among the executive CAC members that the 300 mg/kg dose may be too high for the male rats in the carcinogenicity study.

I supplied the executive CAC members with the following additional information. The trend for body weight gain seen in the 13 week repeat dose rat toxicity study showed a dramatic effect early in the treatment phase with a leveling out of the effect later in the study. The cumulative percent body weight gain decreases for the male and female rats at the end of the 13 week repeat dose thalidomide study were:

A) Male rats: 1) 30 mg/kg - 12.4% decrease, 2) 300 mg/kg - 18.8% decrease and 3) 3000 mg/kg - 31.2% decrease

B) Female rats: 1) 30 mg/kg - 26.7% decrease, 2) 300 mg/kg - 19.4% decrease and 3) 3000 mg/kg - 14.9% decrease

I recommended that the dose selection for male rats be 0, 20, 150 and 300 mg/kg. I recommended that the high dose for the male rats be 300 mg/kg even though the percent body weight gain decrease was 18.8%. This recommendation was based on the body weight curves in the 13 week rat study which showed the 30 and 300 mg/kg dose groups growth curves as parallel curves and the 3000 mg/kg dose group growth curve significantly below that. In addition, I made the executive CAC members aware that I consider this carcinogenicity study to serve as a second species long term toxicity study for thalidomide. Therefore, I informed the executive CAC members that I want to make sure that the highest possible dose is tested in male rats in the carcinogenicity study to not only characterize the carcinogenic potential of thalidomide but also to further characterize the long term toxicity profile for thalidomide in a second species.

I recommended that the dose selection for female rats be 0, 30, 300 and 3000 mg/kg. The basis for this recommendation was because there was not a dose dependent effect on either percent decrease in body weight gain or percent decrease in body weight and that the body weight curves for the low, mid and high dose group female rats are parallel to one another and are slightly, but not significantly, below the control body weight curve.

Based on the additional information provided to the executive CAC members, the executive CAC members concurred with my recommendation to use doses of 0, 20, 150 and 300 mg/kg/day

for male rats and doses of 0, 30, 300, and 3000 mg/kg for female rats. The basis for the dose selection in male rats was the MTD and in female rats was the maximum feasible dose determined in the 13 week toxicity study conducted in rats.

c) Review of protocol for the 104 week rat oncogenicity study

Title: 104 week oncogenicity Study in Rats
Protocol number: not stated
Performing organization: [ ]
Species/Strain: Fischer (F-344) rats (4 weeks old at initiation of dosing)
Number of animals: 70/sex/dose
Duration: 104 weeks
Route: Oral (gavage) at 5 ml/kg/day
Dose Levels: 0 (control; vehicle), 20 mg/kg, 160 mg/kg and 300 mg/kg. The vehicle will be 1% carboxymethyl cellulose aqueous solution.
Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14 and every two weeks thereafter.
5) *Food consumption* - 1X/week for weeks 1-14 and every two weeks thereafter.
6) *Ophthalmology* - performed prior to initiation and at weeks 52 and 104. Both macroscopic and ophthalmoscopic exams of the anterior portion, optic media and ocular fundus will be conducted.
7) *Clinical pathology* - performed at week 52. The first 10 rats/sex/group will be bled and returned to the study. The measurements will consist of total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count, differential leukocyte count, mean corpuscular hemoglobin volume (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).
8) *Unscheduled sacrifices and deaths* - Gross necropsies will be conducted on all moribund animals and on all animals not surviving to termination.
9) *Gross necropsy* - performed at study termination which includes examination of the external surface of the body, all orifices (including the nasal and paranasal sinuses), the cranial, thoracic, and abdominal cavities and their contents.
10) *Organ weights* - performed at study termination and the following tissues will be weighed wet: adrenals, brain, heart, kidney, liver, ovaries, testes, and thyroid/parathyroid (weighed post-fixation).

11) *Histopathological examination* - The following tissues from each necropsied animal will be preserved in 10% neutral buffered formalin: adrenals, aorta, bone with bone marrow (sternum), bone marrow smear, brain (3 levels-fore, mid and hind), eye including optic nerve (2), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), gonads (ovary {2} and testis with epididymides {2}), gross lesions, harderian gland, heart, kidney (2), larynx, liver (all lobes examined), lung with bronchi (2), lymph nodes (mandibular, mesenteric, mediastinal and regional when applicable), mammary gland (female only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular/sublingual, submaxillary), sciatic nerve, skeletal muscle (quadriceps), skin, spinal cord (cervical, thoracic, lumbar), spleen, thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus (both horns and cervix, and vagina . All of the tissues from all dose groups and animals found dead or sacrificed as moribund will be examined microscopically.

12) *Statistical evaluation (as deemed appropriate)* - performed for body weights, food consumption, hematology, organ weights and organ/body and brain weight ratios, incidence of histopathologically proven tumors and survival data.

Comments: The sponsor submitted the first version of the rat oncogenicity study with the NDA submission. The sponsor has incorporated all of the recommendations made for the first version with this second protocol. Therefore, I concur with the protocol for the rat oncogenicity study submitted by the sponsor.

Discussion:

The sponsor was able to submit adequate information to address the concern of cataracts/corneal crystals in the 14 day and 90 day repeat dose mouse studies. I concur with the assessment that the corneal crystals observed in the 90 day study may be due to an age related response rather than a treatment related effect. Also, the concept that the observed cataracts in the 14 day study could be due to their being present pretreatment without being noticed and that a more intensive examination for them at the end of the 14 day study was able to detect them and that this was probably not a treatment related effect is a plausible one.

The sponsor provided evidence that the CD-1 mice used in the 90 day repeat dose toxicity study were healthy and that the alveolar-bronchiolar adenoma and uterine stromal polyp were probably not due to a murine virus infection. However, the cause of these two findings is still unclear. The sponsor provided 10 year old historial data that these two types of lesions are a relatively common spontaneous occurrence in longer term studies (i.e. 18 and 24 month studies). The appropriateness of this data for the current study is questionable. In addition, the sponsor provided evidence that the alveolar-bronchiolar adenoma lesion was a relatively common spontaneous lesion in 6 month studies in CD-1 mice. However, the evidence that the sponsor provided also showed that the uterine stromal polyp was not a spontaneous lesion finding in studies of 6 month or less duration and that the alveolar-bronchiolar adenoma lesion was not a spontaneous lesion finding in studies of 90 days or less. Therefore, the sponsor relied on the opinion of Dr. C

] Manager of Pathology at [ ] to determine that these two lesions were probably not related to the test article. I still believe that there is the possibility that the two observed lesions seen in the 90 day repeat dose toxicity study in mice may be related to test article administration. The conduct of the 2 year carcinogenicity study in CD-1 mice (the protocol for this study was included in the current submission) will help to determine if this is a treatment related finding or not.

The sponsor was able to adequately address the concern about the pharmacokinetic data for the 52 week repeat dose study in dogs. Based on the pharmacokinetic results that the sponsor submitted for the 52 week dog study, I accept the possibility that the difference in AUC values for the 7 day and 52 week dog studies may not be that significant due to the variability of absorption of thalidomide in the gelatin capsules. Since the 7 day study only obtained pharmacokinetic samples from 1 animal/sex/dose group, there does exist the possibility that these values were on the high end of the spectrum. This result does lend support to the importance of obtaining toxicokinetic data in the toxicology animal studies that are conducted with thalidomide due to this large variability in absorption of thalidomide which is probably related to the inherent low water solubility of the test substance.

The information provided by the sponsor to address the concern of the instability of thalidomide under the conditions of the *in vitro* genetic toxicology studies does not alleviate this concern. If anything, the information provided increases the concern. However, the negative result in the *in vivo* genetic toxicology study conducted by the sponsor provides some evidence that thalidomide is not genotoxic. In addition, the sponsor's phase IV carcinogenicity studies in rat and mouse will provide data to determine the potential carcinogenic risk posed by the long term use of thalidomide.

The sponsor submitted revised 2 year rat and mouse carcinogenicity protocols. The sponsor submitted the first version of the mouse oncogenicity study with the NDA submission. The sponsor has incorporated all of the recommendations made for the first version with this second protocol. The only recommendation that I have for this protocol is to modify the dose range for this study. The recommended dose selection for the mouse carcinogenicity study is 0, 100, 1000, and 3000 mg/kg for male and female mice. This dose selection received concurrence from the executive CAC. The high dose selection was based on the maximum feasible dose. The low dose yields an AUC value similar to the highest proposed clinical dose of 400 mg.

The sponsor submitted the first version of the rat oncogenicity study protocol with the NDA submission. The sponsor has incorporated all of the recommendations made for the first version with this second protocol. The only recommendation that I have for this protocol is to modify the dose range for this study. The recommended dose selection for the rat carcinogenicity study is 0, 20, 150, and 300 mg/kg for male rats and 0, 30, 300 and 3000 mg/kg for female rats. This dose selection received concurrence from the executive CAC. The high dose selection was based on MTD for male rats and on the maximum feasible dose for female rats. The rationale for the low dose selection was that this dose yields an AUC value similar to the highest proposed clinical dose of 400 mg.

To address the request for modifications in the reproductive toxicity protocols, the sponsor stated that the reproductive toxicity protocols included in the submission of NDA 20-875 were drafted by [ ] and that the recommendations for modification have been forwarded

to [ ] and are under consideration. The sponsor stated that responses and revised protocols will be submitted within 2 to 3 weeks. A review of the revised reproductive toxicity protocols will be performed when they are submitted to the Agency by the sponsor.

REGULATORY CONCLUSION:

The submitted rat and mouse carcinogenicity protocols are adequate to support the assessment of the carcinogenic potential for thalidomide, provided that the sponsor agrees to the dose selections for both protocols outline below.

RECOMMENDATIONS:

The following information should be relayed to the sponsor:

1) The submitted rat and mouse carcinogenicity protocols are adequate to support the assessment of the carcinogenic potential for thalidomide provided that the sponsor agrees to the dose selections for both protocols outlined below.

2) The following dose selection is recommended for the mouse carcinogenicity study based on maximum feasible dose for male and female mice: 0, 100, 300 and 3000 mg/kg/day. This recommended dose selection has received concurrence from the executive CAC.

3) The following dose selection is recommended for male rats in the rat carcinogenicity study based on MTD: 0, 20, 150 and 300 mg/kg/day. The following dose selection is recommended for female rats in the rat carcinogenicity study based on maximum feasible dose: 0, 30, 300 and 3000 mg/kg/day.

4) The sponsor is encouraged to contact the division if clarification is required concerning the dose selection for either the mouse or rat carcinogenicity studies.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

Agree
Mwleintraub 1/21/98

cc:
NDA: 20-785 (BP)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/PM/WALLING
C:WPFILES/NDAS/NDA20785/207850bp.WPD

Concurrence Only:
HFD-540/OD/WEINTRAUB MW 1/21/98
HFD-540/TOX/AJACOBS 1/15/98

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

IND#: 48,177

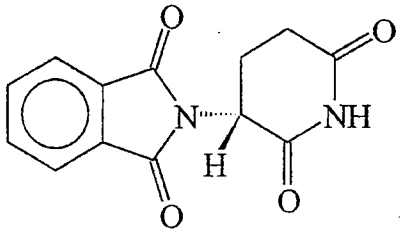
Serial Number: 055

Reviewer: Barbara Hill

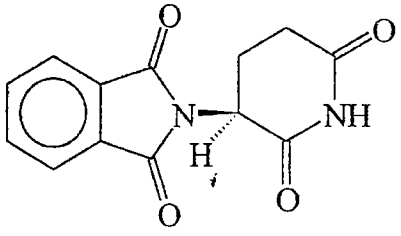
Date CDER Received: 5-11-98
Date Assigned: 5-14-98
Date Review Completed: 5-20-98
Date Accepted by Supervisor: 5-21-98

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, – Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Suppression of Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules

Dose: 50 to 400 mg/day

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [
3) IND
4) IND
5) IND
6) NDA
]
7) NDA 20-785 (Celgene - Thalidomide for ENL)

NOTE: These are just a very small subset of the available INDs that have been submitted to the agency for a variety of indications. The majority of IND submissions for thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains the final oncogenicity protocols to be conducted in mice and rats. The final oncogenicity protocols reflect the thalidomide dose levels that were recommended by the Executive Carcinogenicity Assessment Committee (Exec CAC) received by the sponsor via FAX on 2-17-98.

Review of protocol for the 104 week oncogenicity study in mice:

Title: 104 week Oncogenicity Study of (±) Thalidomide in Mice
Protocol number: Not stated
Performing organization: [ ]
Species/Strain: CD-1 mice (4 weeks old at initiation of dosing)
Number of animals: 80/sex/dose
Duration: 104 weeks
Route: Oral (gavage) at 10 ml/kg/day

Dose Levels: 0 (control; vehicle), 100 mg/kg/day, 1000 mg/kg/day and 3000 mg/kg/day. The vehicle will be 1% carboxymethyl cellulose aqueous solution.

Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14; once every 4 weeks thereafter; at week 104; and at termination.
5) *Food consumption* - 1X/week for weeks 1-14; and every two weeks thereafter.
6) *Ophthalmology* - performed prior to initiation and at weeks 52 and 104. Both macroscopic and ophthalmoscopic exams of the anterior portion, optic media and ocular fundus will be conducted.
7) *Clinical pathology* - performed at week 52. There will be 10 mice/sex/group removed for terminal bleeding and discarded without necropsy. The measurements will consist of total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count, differential leukocyte count, mean corpuscular hemoglobin volume (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).
8) *Unscheduled sacrifices and deaths* - Gross necropsies will be conducted on all moribund animals and on all animals not surviving to termination.
9) *Gross necropsy* - performed at study termination which includes examination of the external surface of the body, all orifices (including the nasal and paranasal sinuses), the cranial, thoracic, and abdominal cavities and their contents.
10) *Organ weights* - performed at study termination and the following tissues will be weighed wet: adrenals, brain, heart, kidney, liver, ovaries, testes, and thyroid/parathyroid (weighed post-fixation).
11) *Histopathological examination* - The following tissues from each necropsied animal will be preserved in 10% neutral buffered formalin: adrenals, aorta, bone with bone marrow (sternum), bone marrow smear, brain (3 levels-fore, mid and hind), eye including optic nerve (2), gallbladder, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), gonads (ovary {2} and testis with epididymides {2}), gross lesions, harderian gland, heart, kidney (2), larynx, liver (all lobes examined), lung with bronchi (2), lymph nodes (mandibular, mesenteric, mediastinal and regional when applicable), mammary gland (female only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular/sublingual, submaxillary), sciatic nerve, skeletal muscle (quadriceps), skin, spinal cord (cervical, thoracic, lumbar), spleen, thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus (both horns and cervix, and vagina. All of the tissues from all dose groups and animals found dead or sacrificed as moribund will be examined microscopically.

12) *Statistical evaluation (as deemed appropriate)* - performed for body weights, food consumption, hematology, organ weights and organ/body and brain weight ratios, incidence of histopathologically proven tumors and survival data.

Comments: The previous protocol for the mouse oncogenicity study was submitted by the sponsor on 11-4-97. This protocol was presented to the Exec CAC on 12-9-97. The original proposed doses for this carcinogenicity study by the sponsor were 0, 100, 1050 and 2000 mg/kg. The Exec CAC recommended using doses of 0, 100, 1000, and 3000 mg/kg where 3000 mg/kg is a maximum feasible dose. The sponsor has incorporated the recommended dose range into the current carcinogenicity protocol. Therefore, this version of the submitted carcinogenicity protocol for thalidomide in mice is acceptable.

Review of protocol for the 104 week rat oncogenicity study:

Title: 104 week Oncogenicity of (±) Thalidomide Study in Rats
Protocol number: not stated
Performing organization: [redacted]
Species/Strain: Fischer (F-344) rats (4 weeks old at initiation of dosing)
Number of animals: 70/sex/dose
Duration: 104 weeks
Route: Oral (gavage) at 5 ml/kg/day
Dose Levels: 0 (control; vehicle), 20 mg/kg, 160 mg/kg and 300 mg/kg for male rats. 0 (control; vehicle), 30 mg/kg, 300 mg/kg and 3000 mg/kg for female rats. The vehicle will be 1% carboxymethyl cellulose aqueous solution.
Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14 and every four weeks thereafter.
5) *Food consumption* - 1X/week for weeks 1-14 and every four weeks thereafter.
6) *Ophthalmology* - performed prior to initiation and at weeks 52 and 104. Both macroscopic and ophthalmoscopic exams of the anterior portion, optic media and ocular fundus will be conducted.
7) *Clinical pathology* - performed at week 52. The first 10 rats/sex/group will be bled and returned to the study. The measurements will consist of total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count, differential leukocyte count, mean corpuscular hemoglobin volume (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).
8) *Unscheduled sacrifices and deaths* - Gross necropsies will be conducted on all moribund animals and on all animals not surviving to termination.

9) *Gross necropsy* - performed at study termination which includes examination of the external surface of the body, all orifices (including the nasal and paranasal sinuses), the cranial, thoracic, and abdominal cavities and their contents.

10) *Organ weights* - performed at study termination and the following tissues will be weighed wet: adrenals, brain, heart, kidney, liver, ovaries, testes, and thyroid/parathyroid (weighed post-fixation).

11) *Histopathological examination* - The following tissues from each necropsied animal will be preserved in 10% neutral buffered formalin: adrenals, aorta, bone with bone marrow (sternum), bone marrow smear, brain (3 levels-fore, mid and hind), eye including optic nerve (2), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), gonads (ovary {2} and testis with epididymides {2}), gross lesions, harderian gland, heart, kidney (2), larynx, liver (all lobes examined), lung with bronchi (2), lymph nodes (mandibular, mesenteric, mediastinal and regional when applicable), mammary gland (female only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular/sublingual, submaxillary), sciatic nerve, skeletal muscle (quadriceps), skin, spinal cord (cervical, thoracic, lumbar), spleen, thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus (both horns and cervix, and vagina . All of the tissues from all dose groups and animals found dead or sacrificed as moribund will be examined microscopically.

12) *Statistical evaluation (as deemed appropriate)* - performed for body weights, food consumption, hematology, organ weights and organ/body and brain weight ratios, incidence of histopathologically proven tumors and survival data.

Comments: The previous protocol for the rat oncogenicity study was submitted by the sponsor on 11-4-97. This protocol was presented to the Exec CAC on 12-9-97. The original proposed doses for this carcinogenicity study by the sponsor were 0, 20, 160 and 300 mg/kg for both male and female rats. The Exec CAC recommended using doses of 0, 20, 150 and 300 mg/kg for male rats based on body weight and body weight gain decreases. The Executive CAC recommended using doses of 0, 30, 300 and 3000 mg/kg for female rats based on maximum feasible dose. The sponsor has incorporated the recommended dose range into the current carcinogenicity protocol. The only exception is that the sponsor listed the doses for male rats as 0, 20, 160 and 300 mg/kg instead of 0, 20, 150 and 300 mg/kg. I do not anticipate that there would be much difference between the results observed at a dose of 150 mg/kg vs 160 mg/kg in male rats. Therefore, this version of the submitted carcinogenicity protocol for thalidomide in rats is acceptable.

REGULATORY CONCLUSION:

The revised mouse and rat oncogenicity protocols appear to be adequate. There is no recommended regulatory action at this time from a pharmacology/toxicology perspective.

RECOMMENDATIONS:

The following information should be relayed to the sponsor concerning IND 48,177 (Serial #055):

1) The revised mouse and rat oncogenicity protocols appear to be adequate.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
IND: 48,177 (055)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/MO/VAUGHAN
HFD-540/CHEM/DECAMP
HFD-105/PM/WALLING
C:/WPFILES/INDS/IND48177/48177055.WPD

Concurrence Only:
HFD-105/OD/WEINTRAUB MW 5/14/97
HFD-540/TOX/AJACOBS 5/14/97

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

NDA#: 20-785

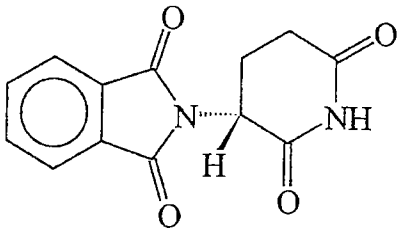
Type of Submission: BP

Reviewer: Barbara Hill

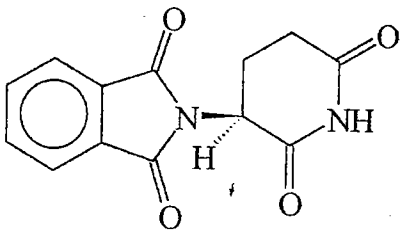
Date CDER Received: 1-7-98
Date Assigned: 1-13-98
Date Review Completed: 3-23-98
Date Accepted by Supervisor: 3-24-98

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	—
[		]
[		]
Stearic Acid NF		]
[		]
[		]
Anhydrous Lactose NF	[	]
Total Fill Weight	400.0	—

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [
3) IND
4) IND
5) IND
6) NDA

]

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for

thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains the final study report for the 52 week toxicity study conducted in dogs. A report based on the 6 month interim sacrifice was included in the original New Drug Application for thalidomide (NDA 20-785) submitted on December 20, 1996. This final report incorporates the earlier results from the interim sacrifice, observations based on the final sacrifice and recovery animals, and toxicokinetic data for Days 1, 178 and 364.

Review of Nonclinical Toxicology Study:

Study Title: A 52 week oral toxicity study of thalidomide in beagle dogs

Study Number: 96583; Performing Organization: [ ] Date Study Completed: 1-5-98; Drug Lot Number: Thalidomide - Lot# 574-574-96-001, 574-574-96-002, 574-574-96-003 and 574-574-96-006; Animal Strain: Beagle dog (8-10 months); Number of Animals: 8/sex/group for control and high dose groups, 6/sex/group for low and mid dose groups (56 total); Dose: 43, 200 or 1,000 mg/kg thalidomide as a dry powder loaded into [ ], size 11 gelatin capsules; Route of Administration: oral; vehicle control - empty [ ] size 11 gelatin capsules; Duration: 52 weeks; GLP Study: Yes

The purpose of this study was to assess the systemic toxicity of thalidomide given as single daily oral doses in capsules to dogs for a period of twelve months. This study was divided into three phases: 1) an interim sacrifice at six months of 2 dogs/sex/group; 2) a terminal sacrifice at 12 months of 4 dogs/sex/group; and 3) a sacrifice after a one month recovery period of 2 dogs/sex/group in the control and high dose groups.

The dose range for the 1 year dog study was selected on the basis of the results of the 1 week pharmacokinetic study in the dog. This study indicated that repeated administration of thalidomide *via* capsule produced increasing drug exposure in response to increasing doses through 1000 mg/kg/day, but no further increase in exposure at 2000 mg/kg/day, probably due to limitations in absorption. Hence, 1000 mg/kg/day was selected as the highest dose in the 1 year dog study. From extrapolation of plasma data from the lowest doses in the dog study and published data measuring plasma levels of thalidomide in healthy male volunteers (Chen, T.L., Vogelsang, G.B., Petty, B.G., Brundrett, R.B., Noe, D.A., Santos, G.W., and Colvin, O.M. 1989. Plasma pharmacokinetics and urinary excretion of thalidomide after oral dosing in healthy male volunteers. Drug Metabol. Dispos. 17: 402-405), it was estimated that a daily dose of 43 mg/kg would produce a similar exposure of thalidomide in the dog as a human receiving a single daily oral dose of 400 mg. The 200 mg/kg dose was selected as a logarithmic midpoint between the 43 mg/kg and 1000 mg/kg dose levels.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, physical examinations (including rectal temperature, respiration rate and electrocardiography), neurological examination (including cerebral, cerebellar, cranial nerves, proprioception, posture and gait, reflexes and sensation), nerve conduction, clinical pathology (hematology and serum chemistry), urinalysis, thyroid function (analysis of TSH, T3 and T4 levels), endocrine function (analysis of prolactin, estradiol, cortisol, corticosterone, aldosterone and ACTH levels). A gross necropsy was performed with measurement of organ weights (brain, heart, liver, thymus, kidneys, adrenal glands, gonads, thyroid, epididymides, spleen and uterus). Representative samples from all gross lesions and from a full spectrum of organs and tissues (including sciatic and sural nerves) were preserved for histopathological evaluation.

Toxicokinetic analysis was performed in this study. Duplicate blood samples were collected from all dogs by jugular venipuncture on Day 1, Day 178 (week 26) and Day 364 (week 52) at 0, 1, 2, 4, 6, 8 and 24 hours post dose administration. Plasma samples were processed according to Eriksson, et. al., 1992. Samples collected on Day 1 were analyzed for drug levels by [redacted] Samples collected on Day 178 and 364 were sent to Celgene for analysis of drug levels. The results of these analyses were presented in this report.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. The primary clinical signs noted during the study period were green colored urine, unchanged test article in the feces and enlarged mammary tissue. Green urine was intermittently observed for 11/12 low dose animals, and all of the mid and high dose animals. The time of onset of discolored urine was governed by dose with the high dose animals first producing green urine at an earlier point during the study (as early as Day 1) than the low and mid dose animals (as late as Day 91). In addition, green urine was intermittently observed for all high dose recovery dogs for 11 to 24 days after the last thalidomide dose. What appeared to be unchanged test article was intermittently observed as a white residue in the feces of 9/12 dogs in the low dose group and all the mid and high dose animals during the first six months of treatment. The presence of test article in the feces was observed with greater frequency with increasing dose. Following the six month interim sacrifice white residue was observed in the feces of 3/8 remaining low dose animals and in all surviving mid and high dose dogs. White residue in the feces was not observed for any animal during the recovery phase portion of this study.

Slight to moderate enlargement of the mammary tissue was observed in 2/6 low dose and mid dose female dogs and 5/8 high dose female dogs. The duration of this enlargement varied from 5 to 144 days and generally was noted to begin just prior to estrus, during estrus or within the two months following estrus. A light blue coloration of the area surrounding the nipples was observed concomitantly with mammary enlargement in five of the nine affected females. This blue coloration was also seen in one control and one high dose dog in which mammary enlargement was not observed at any point during the study. The contract lab suggests that this occurrence in a single control dog suggests that the increased incidence of blue coloration seen in the dosed females may be an exaggeration of normal physiological phenomenon. Mammary enlargement or blue discoloration were not observed during the recovery period. The qualitative aspects of the observed estrous cycles, such as external signs and behavior, were normal for all dogs however the duration of the estrus of heat periods tended to lengthen with increasing thalidomide dose. A

statistically significant difference in mean estrus duration was observed for the high dose females (21 ±9 days) as compared with control (13 ±4 days).

No microscopic changes were seen in the mammary tissue of the dogs necropsied at the interim sacrifice. However, a test article related effect was observed in the mammary glands of females administered thalidomide at the twelve month timepoint. Dilatation of the ducts with distention of the ducts by eosinophilic proteinaceous fluid and/or hyperplasia of the glandular epithelium was observed at all dose levels and ranged from minimal to moderate in severity.

No treatment related effects were seen on body weights, food consumption, ophthalmic exam, physical examinations, neurological examination, nerve conduction, clinical pathology, urinalysis, thyroid function, endocrine function or organ weights. Significant bradycardia was observed in the mid and high dose female dogs during the week 52 measurement. Male dogs in the mid and high dose groups showed a decrease in heart rate at week 52, but this was not significantly different from control animals. Bradycardia was not observed at the interim (6 month) measurement. The bradycardia observed in this study correlates with what is observed in some humans after thalidomide administration.

Yellow-green discoloration of the cranial bones was observed in 2/4 mid dose dogs and 3/4 high dose dogs at the six month interim sacrifice timepoint. One of the affected high dose animals exhibited similar discoloration of the rib, femur and orbit. Yellow discoloration of the femur, skull and/or rib was observed for 5/8 high dose dogs and 1/8 dogs from each of the low and mid dose groups at the twelve month sacrifice timepoint. Discoloration of the bones was not observed in any of the control animals. These findings suggest a test article related effect. However, there was no microscopic correlate present for this finding.

A test article related accumulation of bile plugs in the canaliculi of the liver was observed in all high dose male dogs at the twelve month sacrifice timepoint. This lesion was not seen in the high dose female dogs or in any other dose group. No striking difference was observed between thalidomide dosed and control animals for the mean myelin thickness, mean nerve area (myelin plus axon), mean axonal area and mean myelin area for all measured nerves at the six month interim sacrifice timepoint. Two high dose males displayed slightly less prominent neurofilaments in the axoplasm and a very slight dilation of the axon cylinder when compared to control animals. It was believed by the contract lab that these alterations were probably related to processing artifacts rather than a treatment related effect. This bore out to be a valid assessment since the morphometric and pathologic evaluation of ultrathin sections of the sural nerve and distal spinal cord revealed no test article related ultrastructural morphologic differences between treated and control tissues at the twelve month sacrifice timepoint.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1, 178 and 364 in Beagle dogs are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml) Mean ± SD			T_{max} (hr) Mean ± SD			$T_{½}$,e (hr) Mean ± SD			$AUC_{0\text{-}24hr}$ (µg•hr/ml) Mean ± SD		
		Day 1	Day 178	Day 364	Day 1	Day 178	Day 364	Day 1	Day 178	Day 364	Day 1	Day 178	Day 364
43*	M	2.0± 0.7	2.9± 0.5	2.7± 0.7	2.8± 2.6	2.0± 0.0	1.8± 0.5	2.7± 0.3	2.0± 0.5	2.1± 0.6	14.7± 12.1	16.9± 3.1	18.5± 13.7
43*	F	2.4± 1.7	3.7± 1.5	2.1± 0.5	1.8± 1.1	2.0± 0.0	0.8± 0.5	3.6± 3.5	2.0± 1.3	9.6± 11.6	7.8± 5.8	17.2± 6.4	16.1± 13.4
200*	M	2.6± 0.6	4.3± 1.5	3.5± 0.6	3.0± 1.1	2.7± 1.0	2.0± 0.0	3.9± 2.5	7.5± 6.4	4.4± 2.7	26.6± 16.3	37.5± 9.6	24.7± 16.0
200*	F	5.0± 2.7	7.1± 3.1	4.4± 0.8	5.5± 9.1	3.0± 1.7	2.5± 1.0	2.0± 0.9	2.5± 0.9	7.2± 11.0	49.2± 49.0	64.8± 41.4	41.3± 34.3
1000#	M	7.9± 3.9	15.2±1 .9	9.5± 1.8	6.8± 7.4	4.8± 2.1	2.7± 1.6	21.7± 23.6	5.2± 3.8	16.5± 35.1	104.5± 61.0	179.1± 57.0	82.3± 39.4
1000#	F	8.6± 3.2	15.4± 4.6	9.0± 1.9	11.8± 10.2	3.3± 1.5	2.5± 1.2	7.3± 5.1	7.6± 7.5	30.9± 46.6	112.1± 74.5	146.8± 55.10	97.8± 37.5

* - n = 6 dogs for day 1 and 178 values and 4 dogs for day 364 values.
# - n = 8 dogs for day 1 and 178 values and 6 dogs for day 364 values.

Plasma thalidomide concentration increased with elevation in dose. However, this increase was not proportional to dose. As the dose increases, thalidomide concentrations are present in the plasma for a longer period of time. The generally high coefficient of variations in this study indicate inter-animal variation within dose groups. The relatively large variability in absorption of thalidomide is probably related to the inherent low water solubility of the test substance. The AUC's relative to dose suggests a partial saturation of thalidomide absorption at 1000 mg/kg as compared to lower doses. No clear differences were observed between male and female dog thalidomide plasma pharmacokinetics.

Comments: The daily oral administration of thalidomide to adult Beagle dogs at 43, 200 or 1000 mg/kg/day for one year was generally well tolerated under the conditions of this study. There were no deaths. Test article related changes observed during the study included formation of discolored urine (yellow and/or green), mammary gland enlargement, prolonged duration of estrus and bradycardia (high dose males at twelve month timepoint only). Dilation of mammary ducts, hyperplasia of mammary glandular epithelium and bile plugs in liver canaliculi were postmortem microscopic findings attributed to thalidomide administration. Yellow green discoloration of the bone was observed in thalidomide dosed dogs at necropsy. No microscopic correlate was

observed for this effect. Morphometric and pathologic evaluation of ultrathin sections of the sural nerve and distal spinal cord revealed no test article related ultrastructural morphologic changes in these tissues. It is unfortunate that the administration of thalidomide had no effect on sensory nerve conduction velocity in this study. This result suggests that dogs are not a good animal model for the peripheral neuropathy that is observed in humans after thalidomide therapy.

REGULATORY CONCLUSION:

The submitted one year repeat dose dog study final report is adequate to support the nonclinical development of thalidomide.

RECOMMENDATIONS:

The following information should be relayed to the sponsor:

1) Please submit the final study report for the 52 week toxicity study conducted in dogs to the IND (IND 48,177) for proper tracking purposes.

2) Please submit any additional nonclinical studies for thalidomide to both the NDA (NDA 20-785) and IND (IND 48,177) for proper tracking purposes.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 20-785 (BP)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/PM/WALLING
C:WPFILES/NDAS/NDA20785/20785bp2.WPD

Concurrence Only:
HFD-540/OD/WEINTRAUB MW 3/15/95
HFD-540/TOX/AJACOBS

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

NDA#: 20-785

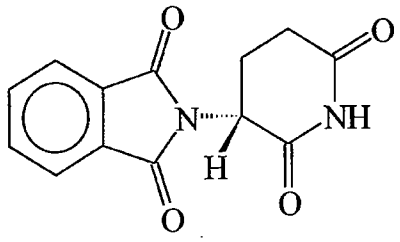
Type of Submission: AP

Reviewer: Barbara Hill

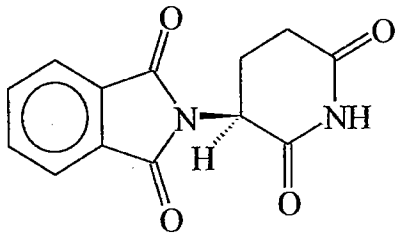
Date CDER Received: 1-9-98
Date Assigned: 1-15-98
Date Review Completed: 2-4-98
Date Accepted by Supervisor: 2-5-98

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	—
[redacted]		
[redacted]		
Stearic Acid NF		
[redacted]		
[redacted]		
Anhydrous Lactose NF		
Total Fill Weight	400.0	—

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [redacted]
3) IND
4) IND
5) IND
6) NDA

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for

thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains the sponsor's response to toxicology concerns that were raised in an approvable letter sent to the sponsor for NDA 20-785 dated September 19, 1997.

Review of sponsor's responses to toxicology concerns raised in the approvable letter:

Note: The original request stated in the approvable letter will be provided followed by a review of the sponsor's response for each toxicology concern.

8. We recommend that you include full hematological and clinical chemistry profile measurements in both of the reproductive toxicity dose range finding studies in male and female rabbits at appropriate time points in this study (i.e., day 7 and study termination). We recommend that you evaluate mating performance in the reproductive toxicity dose range finding study in male rabbits.

The sponsor stated that full clinical laboratory assessments have been added to the dose ranging studies as requested, as has an evaluation of male mating performance. The protocols to be conducted in females and in males were included with the submission and will be reviewed below.

9. We recommend that you evaluate mating performance in the Segment I reproductive toxicity study in rabbits.

The sponsor stated that an evaluation of mating performance has been added to the Segment I reproductive toxicity study. The protocol was included with the submission and will be reviewed below.

10. We recommend that you evaluate a measure of sexual maturation in the Segment III reproductive toxicity study in rabbits. It is also recommended that you evaluate some parameters of development in this study (i.e., measurements of learning capacity, physical strength, and motor coordination).

The sponsor states that the contract lab [ ] has developed a method for evaluating the viability and growth of the offspring of rabbits with minimal loss of kits and/or cannibalization of the kits by the doe. According to the contract lab, rabbit kits are much more sensitive to any disturbance during this stage compared to rat pups. Sexual maturation indices for the F1 generation have been added to the protocol.

The contract lab states that the only parameters not routinely examined in rabbits as they are in rats are evaluations of learning and memory. The contract lab conducted a literature search (Medline 1965 to present) and stated that this search reveals no tests of memory and/or learning of the type typically used for rats have been adapted for rabbits. However, the sponsor also states that the lack of any specific test for learning and/or memory should not preclude the use of the rabbit for evaluation of postnatal functions because the number of other behaviors and functions that can be followed after *in utero* and/or lactational exposure provide a very adequate assessment of normal development. The sponsor states that the protocol as designed will provide enough data to allow an adequate interpretation of the results.

The protocol was included with the submission and will be reviewed below.

11. We recommend that you resubmit the Segment I and III reproductive toxicity protocols after completion of the reproductive toxicity dose range finding studies to support the dose selection for these studies.

The sponsor stated that Celgene Corporation agrees to re-submit the protocols once the dose range finding studies have been completed.

Review of reproductive toxicity protocols and corresponding dose range finding studies:

1) Reproductive toxicity dose range finding study in male rabbits

Title: Oral (stomach tube) Dosage-Range Study of ± Thalidomide in Female Rabbits
Protocol number: 2103-001P
Performing organization: [redacted]
Species/Strain: Female New Zealand White Rabbits (5-7 months; 2.5 - 5.5 kg)
Number of animals: 5/dose
Route: Oral (gavage)
Dose Levels: 0 (control; vehicle - 10 ml/kg/day); 30 mg/kg/day (10 ml/kg of 3 mg/ml stock); 150 mg/kg/day (10 ml/kg of 15 mg/ml stock); 300 mg/kg/day (10 ml/kg of 30 mg/ml stock); and 500 mg/kg/day (10 ml/kg of 50 mg/ml stock). The vehicle will be 1% carboxymethylcellulose aqueous solution. The dose levels were selected by the sponsor on the basis of published results of developmental toxicity studies of thalidomide in the rabbit.
Dosing Schedule: Female rabbits will be given the test article once daily beginning 14 days before artificial insemination (female rabbits will be artificially inseminated using spermatozoa from a single proven male breeder) and continue until the day before sacrifice on day 34 of presumed gestation (female rabbits that do not deliver a litter) or on day 7 postpartum (female rabbits that deliver a litter). Note: F1 generation pups will not be directly given the test article but may be possibly exposed to the test article during maternal

gestation (*in utero* exposure) or via maternal milk during the lactation period.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day
3) *Body weights* - 1X/day
5) *Food consumption* - 1X/day
6) *Hematology and Clinical Chemistry* - A full panel of hematological and clinical chemistry parameters will be obtained for each animal at the scheduled sacrifice.
7) *Toxicokinetics* - Toxicokinetic blood samples will be collected on the first day of dosing and on days 8 and 19 of gestation. On the first day of dosage, samples will be collected at ~1, 2, 4, 8 and 16 hours postdosage. On days 8 and 19 of gestation, samples will be collected immediately prior to dosage, and at ~1, 4, 8 and 16 hours postdosage.
8) *Duration of gestation* - The duration of gestation will be calculated from day 0 of presumed gestation to the day of delivery for the first pup.
9) *Fertility parameters* - The following measurements will be obtained for rabbits assigned to natural delivery: Fertility Index (percentage of matings that result in pregnancies), Gestation Index (percentage of pregnancies that result in birth of live litters), number of offspring per litter (live and dead kits), number of implantation sites, general condition of doe and litter during the postpartum period, viability indices (percentage of kits born that survive 4 and 7 days).
10) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.
11) *Female rabbit necropsy* - Female rabbits will be sacrificed after completion of the seven day postpartum period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. The number and distribution of implantation sites will be recorded for all female rabbits. Dams with no surviving kits will be sacrificed after the last kit is found dead or missing. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for each female rabbit.
12) *F1 generation measurements* - Litters will be observed for dead pups at least twice daily. Clinical observations will be conducted on postpartum days 4 and 7. Body weights will be measured on postpartum days 4 and 7. Kits will be sacrificed on postpartum day 7 and examined for gross lesions. Necropsy will include a single cross-section of the head at the level of the frontal parietal suture and examination of the cross sectioned brain for apparent hydrocephaly. Kits that die prior to postpartum day 7 will be examined to try to determine the cause of death.

Comments: The protocol outlined for the dose range study appears to be adequate. ✓

2) Reproductive toxicity dose range finding study in male rabbits

Title: Oral (stomach tube) Dosage-Range Study of ± Thalidomide in Male Rabbits
Protocol number: 2103-002P
Performing organization: [ ]
Species/Strain: New Zealand White Rabbits (5-7 months; 2.5 - 5.5)
Number of animals: 5/sex/dose (only male rabbits will receive the test article)
Route: Oral (gavage)
Dose Levels: 0 (control; vehicle - 10 ml/kg/day); 30 mg/kg/day (10 ml/kg of 3 mg/ml stock); 150 mg/kg/day (10 ml/kg of 15 mg/ml stock); 300 mg/kg/day (10 ml/kg of 30 mg/ml stock); and 500 mg/kg/day (10 ml/kg of 50 mg/ml stock). The vehicle will be 1% carboxymethylcellulose aqueous solution. The dose levels were selected by the sponsor on the basis of published results of developmental toxicity studies of thalidomide in the rabbit.
Dosing Schedule: Male rabbits will be given the test article once daily beginning 14 days before mating and continue until the day before sacrifice (after a total of 42 days of dosing). Female rabbits will not be given the test article.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day for male rabbits; Days 0, 6, 13, 18 and 20 of presumed gestation for female rabbits.
3) *Body weights* - 1X/day for male rabbits; Days 0, 6, 13, 18 and 20 of presumed gestation for female rabbits.
5) *Food consumption* - 1X/day
6) *Hematology and Clinical Chemistry* - A full panel of hematological and clinical chemistry parameters will be obtained for each animal at the scheduled sacrifice.
7) *Toxicokinetics* - Toxicokinetic blood samples will be collected on the first and last day of dosage from all male rabbits. On the first day of dosage, samples will be collected at ~1, 2, 4, 8 and 16 hours postdosage. On the last day of dosage, samples will be collected immediately prior to dosage, and at ~1, 4, 8 and 16 hours postdosage.
8) *Caesarean-sectioning observations* - Female rabbits will be Caesarean-sectioned on day 20 of presumed gestation. The rabbits will be examined for the number and distribution of: corpora lutea, implantation sites, viable and nonviable embryos.
9) *Sperm Evaluation* - Samples of semen will be collected from each male rabbit and analyzed for motility and count following test article administration on the last day of dosage (day 28 of dosage).
10) *General necropsy information* - All rabbits will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.

11) *Male rabbit necropsy* - Male rabbits will be sacrificed on the day following the completion of the 28 day dosage period. A gross necropsy of the thoracic, abdominal and pelvic viscera will on performed on all male rabbits. The testes and epididymides will be removed, individual organ weights will be recorded and the organs will be preserved for histological examination. Testes and epididymides of control and high dosage group rabbits will be examined histologically.

12) *Female rabbit necropsy* - Female rabbits will be Caesarean-sectioned on day 20 of presumed gestation and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Comments: The protocol outlined for the dose range study appears to be adequate. ✓

3) Segment I reproductive toxicity study in rabbit

Title: Oral (gavage) Fertility and General Reproduction Toxicity Study of ± Thalidomide in Rabbits

Protocol number: 2103-002

Purpose: The purpose of this study is to test for toxic effects/disturbances resulting from thalidomide treatment of male and female New Zealand White rabbits before mating and through gestation. This study evaluates ICH Harmonized Tripartite Guideline stages A through D of the reproductive process and should detect effects on tubal transport, implantation, and development of the embryos and fetuses of female rabbits and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rabbit reproductive organs.

Performing organization: [ ]

Species/Strain: New Zealand White rabbit (5-7 months; 2.5 - 5.5 kg)

Number of animals: 20/sex/dose

Route: Oral (gavage)

Dose Levels: There will be a control (vehicle), low, mid and high dose group. The dose levels will be selected on the basis of the results of the reproductive toxicity dose range finding studies in rabbit. The vehicle will be 1% carboxymethylcellulose aqueous solution.

Dosing Schedule: Male rabbits will be given the test article once daily beginning 14 days before mating and continue through the day before sacrifice (a total of 56 days of dose administration). Female rabbits will be given the test article once daily beginning 14 days before mating and continue until the day before sacrifice on day 29 of presumed gestation.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 2X/day
3) *Body weights* - 1X/day
4) *Food consumption* - 1X/day
5) *Caesarean-sectioning observations* - Rabbits will be Caesarean-sectioned on day 29 of presumed gestation. Placentae that appear abnormal (size, color or shape) will be noted in the raw data. The rats will be examined for number and distribution of: corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions.
6) *Fetal observations* - Body weight, sex and gross external alterations will be determined for each fetus. Fetuses with gross external alterations will be preserved in neutral buffered 10% formalin. All other fetuses will be discarded after examination.
7) *Semen/Sperm evaluation* - Samples of semen will be collected from each male rabbit after the last day of dosage (day 56 of dosage) and analyzed for sperm motility and count. Samples of semen from each rabbit will be analyzed for the presence of test article.
8) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.
9) *Male rabbit necropsy* - Male rabbits will be sacrificed following the completion of the 56 day dosage period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed on all male rats. The testes and epididymides will be removed, individual organ weights will be recorded and the organs will be preserved for histological examination. Testes and epididymides of control and high dosage group rabbits will be examined histologically.
10) *Female rabbit necropsy* - Female rabbits will be sacrificed on day 29 of presumed gestation. A Caesarean-section will be performed at that time and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rats. The number and distribution of implantation sites will be recorded for each female rabbit. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites. All ovaries will be preserved for possible future evaluation.

Comments: The protocol outlined for the Segment I study appears to be adequate. The sponsor is advised to submit the anticipated doses to be used for this study along with the results of the reproductive toxicity dose range finding studies in support of the dose selection for the Segment I study. ✓

4) Segment III reproductive toxicity study in rabbit

Title: Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of thalidomide in rabbits, including a postnatal reproductive evaluation

Protocol number: 2103-001

Purpose: The purpose of this study is to detect adverse effects of thalidomide treatment of female New Zealand White rabbits from the closure of the hard palate through lactation and weaning on gestation, parturition, lactation and maternal behavior in female rabbits and on the development of the offspring of the treated female rabbits. This study evaluates ICH Harmonized Tripartite Guideline stages D through F of the reproductive process and does not include an evaluation of Caesarean-delivered fetuses (stages C and D), because this evaluation is performed in a supplementary study. Because manifestations of effects induced during this period may be delayed, observations will be continued through sexual maturity of the F1 generation rabbits.

Performing organization: [redacted]

Species/Strain: Female New Zealand White rabbit (5-7 months; 2.5 - 5.5 kg)

Number of animals: 25 mated females/dose

Route: Oral (gavage)

Dose Levels: There will be a control (vehicle), low, mid and high dose group. The dose levels will be selected on the basis of the results of the reproductive toxicity dose range finding studies in rabbit. The vehicle will be 1% carboxymethylcellulose aqueous solution.

Dosing Schedule: Female rabbits will be given the test article once daily from day 18 of presumed gestation through day 28 postpartum or day 33 of presumed gestation (rabbits that do not deliver a litter). F1 generation kits will not be directly given the test article, but may be possibly exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Assessments for Fo Generation:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day
3) *Body weights* - 1X/day
4) *Food consumption* - 1X/day
5) *Milk sample collection* - Milk samples will be collected from female rabbits once ~4 hours postdosage between days 8 and 14 postpartum.
6) *Natural delivery* - Female rabbits will be evaluated for the following criteria during natural delivery: 1) clinical observations (including the time each kit is delivered) during parturition (between 0700 and 1900 hours EST); 2) duration of gestation (day 0 of presumed gestation to the time the first pup is delivered; 3) length of parturition (time of delivery of last kit minus the time of delivery of the first kit divided by N-1 kits in each litter); 4) litter size (defined as all kits delivered); 5) viability indices (percentage of kits born that survive 4 and 7 days); and 6) lactation index (percentage of kits born that survive 28 days).

8) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.
9) *Female rat necropsy* - Female rabbits will be sacrificed after the 28 day postpartum period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. The number and distribution of implantation sites will be recorded for each female rabbit. Female rabbits that do not deliver a litter will be sacrificed on day 34 of presumed gestation and examined for gross lesions. Uteri will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Assessments for F1 Generation:

1) *Viability* - Preweaning period: Litters will be observed for dead kits at least twice daily. The kits in each litter will be counted 1X/day. Postweaning period: 2X/day.
2) *Clinical observations* - Preweaning period: 1X/day. Postweaning period: 1X/week.
3) *Body weights* - Preweaning period: Days 4, 7, 14, 21 and 28 postpartum. Postweaning period: 1X/week. Presumed gestation period: Days 0, 7, 10, 14, 17, 21, 24 and 29 (female rabbits only)
4) *Food consumption* - Preweaning period: not recorded. Postweaning period: 1X/day
5) *Sexual Maturation* - Male rabbits will be evaluated for the age of preputial separation and females for the age of vaginal patency.
6) *Reproductive evaluation* - Twelve male and twelve female rabbits per dose group will be selected for reproductive evaluation at five months of age. One male rabbit and one female rabbit will be paired for mating and each pair will be monitored continuously until mating is confirmed by observation. Following mating, the female rabbit will be returned to it's individual cage. The day of mating will be designated day 0 of presumed gestation. Female rabbits will be Caesarean sectioned on day 29 of presumed gestation. The rabbits will be examined for number and distribution of: corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus will be weighed and examined for sex and gross external alterations. Representative photographs of fetal alterations will be taken. Fetuses will be tagged with identification noting study number, litter number, uterine distribution and fixative, and retained for possible future evaluation.
7) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. Selected F1 rabbits for reproductive evaluation will have sections of the sciatic, tibial, fibular and sural nerves excised and preserved for possible future histological evaluation. All other tissues will be discarded unless specifically cited below.
8) *Kit necropsy* - Kits found dead or sacrificed because of moribundity will be examined for gross lesions and for the cause of death or the moribund condition. Gross lesions will be preserved for possible future evaluation. All kits culled on day 28 postpartum (kits not selected for continued observation) will be sacrificed and examined for gross lesions. The gross lesions will be preserved for possible future evaluation. Necropsy will include a

single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.

9) *Male rabbit necropsy* - Male rabbits will be sacrificed after completion of mating. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Testes and epididymides of male rabbits will be excised and paired organ weights will be recorded and the organs will be preserved for possible future evaluation.

10) *Female rabbit necropsy* - Female rabbits will be sacrificed on day 29 of presumed gestation and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for each rat. Uteri of apparently nonpregnant rabbits will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Comments: The sponsor states that the protocol as designed will provide enough data to allow an adequate interpretation of the results for effects on development. However, it is unclear exactly what behaviors will be assessed for indices of functional development. The ability to examine the effect of the test article on functional development is important because the test article effects on later development are unknown. It is recommended that the sponsor clearly outline at the beginning of the protocol what will be examined for effects of the test article on functional development. This can be accomplished when the study is resubmitted after completion of the dose range studies. Aside from this issue, the protocol outlined for the Segment III study appears to be adequate.

DISCUSSION:

I requested consult advice from David Morse, Ph.D., a pharmacology/toxicology reviewer in the Division of Antiviral Drug Products. David is considered the Center's expert in the area of behavioral tests. I inquired whether the sponsor was correct that functional/behavioral tests are not standardized for testing functional development in a Segment III reproductive toxicity study in rabbits. The reply to this request from David is provided below.

While it is true that the majority of the functional/behavioral tests have been developed in rodents (and subsequent studies are most commonly conducted in the accepted model; i.e., the rodent), most of the tests are equally applicable to non-rodent species. For instance the following tests most commonly used with rodents could easily be conducted in rabbits: a) spontaneous locomotor activity (in an open field), b) righting reflex and tilting plane, c) acoustic startle, d) passive avoidance, e) schedule controlled behavior (operant learning tasks), and f) nerve conduction assays. There are however some tasks that would be inappropriate/inapplicable/difficult to the rabbit, such as: a) hanging wire task, b) most nociception tests (because rabbits have a layer of insulating fur on the footpads), and c) most swimming tests.

At the very least, the sponsor should be performing a FOBS (Functional Observational Battery's; cf, Proc. Symposium Predicting Neurotox & Behav. Dysfunction from PreClin. Tox. Data, in Neurotox. & Teratol., Vol 9, 1987) as part of their Seg III reprotox study(ies). At bare minimum, the sponsor should be attempting to measure some form of spontaneous and reflex activity in the test animals (motor function), along with sensory functioning, learning and memory (cognition). There

is really no reason why this kind of evaluation can't be performed -- particularly when there is reason from the prior human experience to suspect that the compound should demonstrate neurologic activity (i,e, sedation and/or motivational changes, changes in memory and learning, peripheral neuropathy, etc.).

Searching in Medline for literature relevant to rabbit behavioral testing, not unexpectedly, is unlikely to produce much of anything. You would have to search in ToxLine or, even more likely, in PsychInfo for the Journals relevant to these kinds of measures. Most of the relevant material will be located in journals which focus on Animal or Operant Behavioral Testing.

Based on David's response, I now believe that it would be in the best interest of the Agency to request that some behavioral tests be included in the Segment III reproductive toxicity protocol as a measure of functional development. I recommend that, at a minimum, the following tests be included in the protocol: a) spontaneous locomotor activity (in an open field), b) righting reflex and tilting plane, c) acoustic startle, d) passive avoidance, and e) schedule controlled behavior (operant learning tasks). This information will be crucial because we currently do not possess any knowledge of the effects of thalidomide on later stages of development in the fetus. ✓

REGULATORY CONCLUSION:

The submitted reproductive toxicity dose range finding studies in male and female rabbits appear to be adequate. The Segment I reproductive toxicity study protocol appears to be adequate, ✓ but the dose selection for this study will need to be evaluated after completion of the two ✓ reproductive toxicity dose range finding studies. It is unclear how the effect of the test article on functional development will be measured in the submitted Segment III reproductive toxicity study protocol. It is recommended that the sponsor clearly outline at the beginning of the protocol what will be examined for effects of the test article on functional development. This can be accomplished when the study is resubmitted after completion of the dose range studies. It is recommended that, at a minimum, the following tests be included in the protocol: a) spontaneous locomotor activity (in an open field), b) righting reflex and tilting plane, c) acoustic startle, d) ✓ passive avoidance, and e) schedule controlled behavior (operant learning tasks). Aside from this issue, the protocol outlined for the Segment III study appears to be adequate.

RECOMMENDATIONS:

The following information should be relayed to the sponsor:

1) The submitted reproductive toxicity dose range finding studies in male and female rabbits ✓ appear to be adequate.

2) The Segment I reproductive toxicity study protocol appears to be adequate, but the dose ✓ selection for this study will need to be evaluated after completion of the two reproductive toxicity dose range finding studies.

3) It is unclear how the effect of the test article on functional development will be measured in the submitted Segment III reproductive toxicity study protocol. It is recommended that the sponsor clearly outline at the beginning of the protocol what will be examined for effects of the test article on functional development. This can be accomplished when the study is resubmitted after completion of the dose range studies. It is recommended that, at a minimum, the following tests be included in the protocol: a) spontaneous locomotor activity (in an open field), b) righting reflex and tilting plane, c) acoustic startle, d) passive avoidance, and e) schedule controlled behavior (operant learning tasks). Aside from this issue, the protocol outlined for the Segment III study appears to be adequate.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 20-785 (AP)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/PM/WALLING
C:WPFILES/NDAS/NDA20785/207850ap.WPD

Concurrence Only:
HFD-540/OD/WEINTRAUB MW 2/6/98
HFD-540/TOX/AJACOBS 2/5/98

mw 4/98

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

NDA#: 20-785

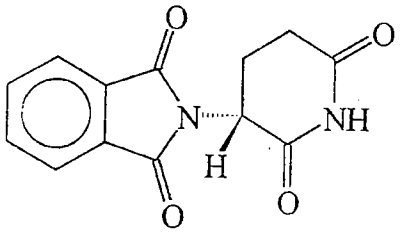
Type of Submission: BL

Reviewer: Barbara Hill

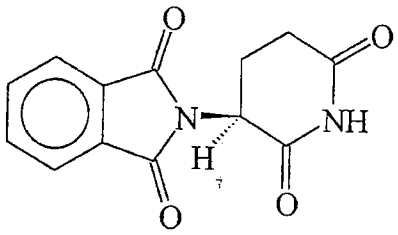
Date CDER Received: 10-21-97
Date Assigned: 11-7-97
Date Review Completed: 12-4-97
Date Accepted by Supervisor: 12-8-97

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Thalomid, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	—
[illegible]		
[illegible]		
Stearic Acid NF		
[illegible]		
[illegible]		
Anhydrous Lactose NF		
Total Fill Weight	400.0	—

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
2) IND [illegible]
3) IND
4) IND
5) IND
6) NDA

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Contents of this submission:

This submission contains a revised label (package insert) for NDA 20-785 [Thalomid (thalidomide)].

Review Objective:

An electronic copy of the revised label was submitted to the agency by the sponsor along with the official paper copy in the jacket. This electronic version is reproduced below and recommendations for revision of the pharm/tox information contained in this label is made directly to the label via addition of red lined text and deletion of striked out text.

Note: The original electronic version was submitted in Word 6.0/7.0. I have copied and pasted the information from the Word document into my review in WordPerfect 6.1. All of the information transferred accurately from the Word document into this review.

PACKAGE INSERT

17 Page(s) Withheld

____ § 552(b)(4) Trade Secret / Confidential

____ § 552(b)(5) Deliberative Process

✓ § 552(b)(4) Draft Labeling

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 20-785 (BL)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/PM/WALLING
C:WPFILES/NDAS/NDA20785/207850bl.WPD

Concurrence Only:
HFD-540/OD/WEINTRAUB MW 4/4/98
HFD-540/TOX/AJACOBS 4/2/98

AA1AC005

Executive CAC
December 9, 1997

Committee: Ron Steigerwalt, Ph.D., HFD-510, Acting Chair
Paul Andrews, Ph.D., HFD-150, Alternate Member
C. Joseph Sun, Ph.D., HFD-570, Alternate Member
Barbara Hill, Ph.D., HFD-540, Presenting Reviewer
Abby Jacobs, Ph.D., HFD-540, Division Team Leader
Wendelyn Schmidt, Ph.D., HFD-024, Project Manager

Author of Draft: Wendelyn Schmidt

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA 20-785
Thalidomide
Celgene Corp.

Background:
Thalidomide is being used as an immunomodulator. No in vitro induction of human microsomes was noted with thalidomide.

Proposed Mouse 2 Year Carcinogenicity Study:
The doses proposed for the two year study are based on a 13 week oral gavage (in 1% carboxymethyl cellulose) study. Doses proposed are 0, 100, 1050, and 2000 mg/kg/day. The 100 mg/kg/day dose approximates the human exposure, while the top dose was based on a presumed saturation of absorption. The measurements for pharmacokinetic determination were made on the parent drug only. The exposure was non-linear, but where saturation occurred was not determined. The committee recommended using the maximum feasible dose (3000 mg/kg/day).

Proposed Rat 2 Year Carcinogenicity Study:
The proposed doses in this study are based on a 13 week toxicity study (doses of 0, 30, 300, 3000 mg/kg) . In the males, an 18.5% decrement in body weight was noted at the highest dose. In the females, body weights were decreased non-dose dependently. Pharmacokinetics were non-linear with dose.

Executive CAC Recommendations and Conclusions:
Mouse study:
The committee recommended using doses of 0, 100, 1000, and 3000 mg/kg/day where 3000 mg/kg is a maximum feasible dose.

Rat:
1) The changes in male body weight should be expressed as body weight gain.
2) Assuming no greater than a 10% decrement in body weight, the recommended doses for the males are 0, 20, 160, and 300 mg/kg.
3) As body weight changes in the females were independent of dose, and AUC does not necessarily show saturation with dose, recommended doses are 0, 30, 300, and 3000 mg/kg.

Addendum (12-31-97):

Additional information was provided to the executive CAC members via email on body weight gain changes for both male and female rats in the 13 week toxicity study. Concurrence on dose selection for the rat carcinogenicity study was reached by all members by 12-29-97. The committee concurred on the following doses for the rat carcinogenicity study:
1) 0, 20, 150 and 300 mg/kg for male rats based on body weight and body weight gain decreases.
2) 0, 30, 300 and 3000 mg/kg for female rats based on maximum feasible dose.

Ronald W. Steigerwalt
Ron Steigerwalt, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, HFD-540, NDA 20-785
/BHill, HFD-540
/AJacobs, HFD-540
/WSchmidt, HFD-024

JUN 12 1997

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

NDA#: 20-785

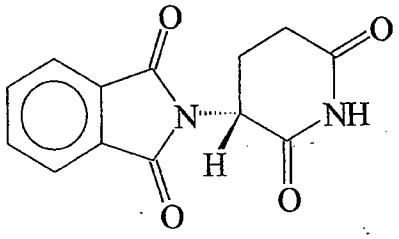
Type of Submission: Original NDA; 1P

Reviewer: Barbara Hill

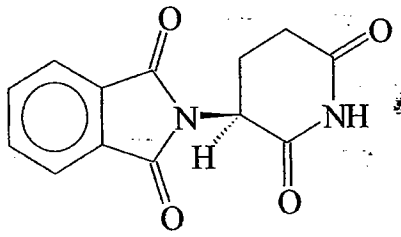
Date CDER Received: 12-23-96
Date Assigned: 12-31-96
Date Review Completed: 6-9-97
Date Accepted by Supervisor: 6-12-97

Name of Drug: Thalidomide, Distaval, Isomin, Kevadon, Rosalon, Sauramide, Sortenon, Talimol, Synovir, α-(phthalimido)glutarimide

Structure: Formulation contains a racemic mixture of both the + and – enantiomers.



R-(+)-Thalidomide



S-(–)-Thalidomide

Molecular Weight: 258.2

Molecular Formula: $C_{13}H_{10}N_2O_4$

Pharmacological Category: Immunomodulator

Sponsor: Celgene Corporation
7 Powder Horn Drive
PO Box 4914
Warren, NJ 07059
(908) 271-4184

Indication: Erythema Nodosum Leprosum (ENL)

Route of Administration: Oral

Formulation: 50 mg hard gelatin capsules (size 0)

Substance	Weight (mg)	% by Weight
Thalidomide	50.0	-
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
Stearic Acid NF	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
Anhydrous Lactose NF	[ ]	[ ]
Total Fill Weight	400.0	-

Dose: 100 to 400 mg/day

The final dosing regimen for thalidomide in the treatment of ENL has not been determined for this NDA at the time of this review.

Related INDs and NDAs:

1) IND 48,177 (Celgene - Thalidomide for ENL)
2) IND [
3) IND [ ]
4) IND 11,359 (Thomas Yoder of Hansen's Disease Center - Thalidomide for treatment of ENL)
5) IND [
6) NDA [ ]

NOTE: This is just a very small subset of the available INDs for thalidomide that have been submitted to the agency for a variety of indications. The majority of IND submissions for

thalidomide are from individual investigators that wish to treat either a single patient or a small number of patients with thalidomide for a particular indication.

Review Objective:

To determine if the submitted non-clinical studies support the safe use of Thalidomide in humans and support the non-clinical labeling section for the Thalidomide NDA from a non-clinical pharmacological/toxicological basis.

Background/Introduction:

Thalidomide was synthesized and marketed by Chemie Grunenthal, Stolberg, Germany, in the 1950s as a sedative. The toxicity of the compound was considered to be extremely low because an LD_{50} could not be established in animal toxicity studies. Therefore, it was believed that thalidomide promised to offer a safe alternative to the use of barbiturates. The observation of polyneuritis associated with long term thalidomide administration (Florence, AL. 1960. Is thalidomide to blame? Lancet 2: 1954; Fullerton, P.M. and Dremer, M. 1961. Neuropathy after intake of thalidomide (Distaval). British Medical Journal 2: 855-858) first suggested that the compound produces serious adverse effects. In 1961 thalidomide was realized to be responsible for an epidemic of malformations (Lenz, W. 1962. Thalidomide and congenital abnormalities. Lancet 1: 45; McBride, W.G. 1961. Thalidomide and congenital abnormalities. Lancet 2: 1358). The incidence of malformed babies paralleled the sales of thalidomide preparations (Lenz W. 1988. A short history of thalidomide embryopathy. Teratology 38: 203-215). These adverse effects caused thalidomide to be withdrawn from the market 30 years ago.

Thalidomide administration during a defined sensitive period (day 35-50 post menstruation) produced a pattern of malformations resembling a combination of the Holt-Oram and Fanconi syndromes in humans. A single dose of thalidomide (100 mg) leading to plasma concentrations of ~1 μg/ml was sufficient to produce typical malformations. The typical spectrum of malformations elicited by thalidomide include phocomelia or amelia of the limbs which followed the craniocaudal progression of morphogenesis. The inner ear and the eyes were frequently affected by thalidomide, but the development of teeth was normal. The skeletal effects were often combined with defects of internal organs (cardiovascular system, duodenum, respiratory tract, urogenital tract, gallbladder and rectum).

Thalidomide has regained favor as a therapeutic agent despite the terrible teratogenic tragedy suffered in the late 50's and early 60's. Sheskin (Sheskin J. 1965. Thalidomide in the treatment of lepra reactions. Clinical Pharmacology and Therapeutics 6: 303-306) was the first to report that thalidomide suppresses ENL, a complication of the lepromatous form of leprosy. This effect was not the consequence of an antibacterial action, but was due to a direct influence on the immune system. During the following years thalidomide was found to be an effective symptomatic treatment for a number of systemic, cutaneous and mucocutaneous disorders. Evidence accumulated that thalidomide was acting as an immunomodulator as opposed to an immunosuppressive agent.

Pharmacologically, thalidomide is a potent central nervous system depressant in some species and exhibits anti-inflammatory and immunomodulatory effects. The biochemical mechanism behind the immunomodulatory effect of thalidomide is unclear. One of the most popular hypotheses is that thalidomide inhibits the formation of tumor necrosis factor alpha (TNFα). It has been demonstrated *in vitro* that thalidomide inhibits TNFα production by accelerating the degradation of the mRNA coding for it (Moreira, A.L., Sampaio, E.P., Zmuidzinas, A., Frindt, P., Smith, K.A. and Kaplan, G. 1993. Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. J. Exp. Med. 177: 1675-1680). Overproduction of TNFα has been associated with a wide variety of disease, including ENL, tuberculosis, malaria, cancer, graft *versus* host disease and HIV-infection. In patients with ENL, control of symptoms with thalidomide has been associated with reduction in agonist-stimulated monocyte TNFα secretion (Barnes, P.F., Chatterjee, D., Brennan, P.J., Rea, T.H. and Modlin, R.L. 1992. Tumor necrosis factor production in patients with leprosy. Infect. Immun. 60: 1441-1446) and reduction of serum TNFα levels (Sampai, E.P., Kaplan, G., Miranda, A., Nery, J.A., Miguel, C.P., Viana, S.M. and Sarno, E.N. 1993. The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. J. Infect. Dis. 168: 408-414).

Thalidomide is an example of a unique drug where the potential toxicities associated with use were determined in humans prior to animals. Despite the number of years that thalidomide has been studied, there is a paucity of nonclinical toxicity data available (with the exception of reproductive toxicology studies) generated under modern protocols and performed under Good Laboratory Practices Regulations. In the current submission, Celgene has completed acute and subchronic toxicity studies and mutagenicity studies to provide a more current and complete data base from which to evaluate the potential for thalidomide to produce toxicity. Celgene has also agreed to conduct several additional nonclinical toxicity studies as a phase IV commitment to further characterize the toxicity profile for thalidomide. The protocols that are contained in this submission are for nonclinical studies to determine the carcinogenic and additional reproductive toxicity potential (in addition to the well characterized teratogenicity) for thalidomide.

The main toxicity of thalidomide is its well-known teratogenicity. Exposure to thalidomide during the sensitive period of pregnancy (days 8 to 10 for rabbits) results in a wide spectrum of malformations in a number of soft and skeletal tissues, but particularly in the limbs. Embryotoxic effects are variable among species and strains, with the mouse and rat being somewhat resistant (or more precisely, responding with increased resorptions), and the rabbit, hamster, and primate being variably responsive, producing live fetuses with characteristic defects. These differences do not appear to be due to species differences in pharmacokinetics. Due to the well characterized teratogenic effects of thalidomide in the literature no additional evaluation of reproductive toxicity has been conducted by Celgene.

Sensory peripheral neuropathy is one of the primary non-teratogenic adverse effects that have been clinically associated with thalidomide. Subsequent investigations of this effect in animals has met with limited success. Functional and morphological neural abnormalities have been reported for rabbits. However, no thalidomide-induced neural defects are reported for a number of other species. Following long-term administration of thalidomide (100 mg/kg/day

p.o.) to the New Zealand white rabbit, decreased nerve conduction velocity was reported in the absence of clinical or morphological abnormalities. Celgene has attempted to expand the knowledge base regarding the neuropathic potential of thalidomide by including Functional Observations Batteries in long-term rat and dog studies. A specific sural nerve morphology evaluation using electron microscopy, and ongoing routine nerve conduction velocity and amplitude testing of the sciatic nerves is also being undertaken in dogs in the 1 year dog study. Celgene has submitted a six month interim report in this NDA package for the 1 year dog study.

Index of Nonclinical Studies:

Nonclinical Pharmacokinetic Studies:

Nonclinical Toxicity and Safety Studies:

Note: Several of the toxicology studies for this NDA were conducted as combined toxicology/pharmacokinetic studies. The sponsor felt that this scenario would be helpful for toxicokinetic purposes to determine the C_{max} or AUC at which a particular toxicity was expressed for thalidomide. Therefore, in the review of the toxicology studies, the pharmacokinetic data will be presented in conjunction with the appropriate toxicology study.

A) Subchronic Toxicology Studies:

B) Genetic Toxicology Studies:

1) Page 37 *Ames/Salmonella-E. coli* reverse mutation assay on thalidomide
2) Page 37 AS52/XPRT mammalian cell forward gene mutation assay on thalidomide
3) Page 38 *In vivo* micronucleus test with thalidomide in mouse bone marrow erythropoietic cells

Note: Celgene took great care with the thalidomide samples used and obtained in the nonclinical studies to compensate for the chemical and physical properties of thalidomide, especially with respect to sample handling. Due to the relative insolubility of thalidomide in aqueous media and the high viscosity of the CMC preparations, dosing suspensions were prepared fresh daily and continuously stirred before and during dosing of the animals. It has been well established in the literature that thalidomide undergoes a rapid rate of hydrolysis when exposed to aqueous media near physiological pH and ambient temperatures. To safeguard against this occurrence in the nonclinical studies, plasma samples taken for toxicokinetic analysis and even CMC suspensions taken for dose analysis and homogeneity were quickly buffered with equal volumes of Sorensen's citrate buffer (pH 1.5) and frozen according to the recommendations reported in the literature (Eriksson, T., Bjorkman, S., Fyge, A. and Ekberg, H. Determination of thalidomide in plasma and blood by high-performance liquid chromatography: Avoiding hydrolytic degradation. J. Chrom., 582, 211-216, 1992). Subsequent thawing and re-suspension of samples was performed rapidly but thoroughly. Analysis of thalidomide samples, regardless of their laboratory of origin, was performed by the same analytical laboratory ([ ] to minimize possible variations in testing. The results of the dose analysis and homogeneity verification from all of the Celgene-sponsored toxicokinetic studies were within acceptable ranges. The data also indicate that doses were prepared correctly in each study and there was no appreciable degradation of thalidomide concentration over time (in the buffered or frozen state in PEG-400, DMSO or as a 1% CMC suspension).

Nonclinical Pharmacokinetic Studies:

1. Pharmacokinetics and bioavailability of thalidomide following single oral or intravenous dose administration of thalidomide in male mice

Study Number: 841-CEL-001-95; Performing Organization: [ ] Date Study Completed: 11-19-96; Drug Lot Number: Thalidomide, Lot# 574-574-95-001; Animal Strain: male CD-1 mice (53 days; 25-34 g); Number of Animals: 6/dose/timepoint; Dose: oral - 200, 1000 or 2,000 mg/kg in 1% CMC, iv - 10 mg/kg in PEG-400; Route of Administration: oral (gavage) or intravenous; Vehicle - 1% CMC (oral) and PEG-400 (iv); Dose Volume: oral - 10 ml/kg of 20, 100 and 200 mg/ml stock, iv - 4 ml/kg of 2.5 mg/ml stock; Duration: 72 hours; GLP Study: Yes.

The purpose of this study was to determine the plasma pharmacokinetics and bioavailability of thalidomide following single oral or intravenous dose administration of thalidomide in male mice. The only toxicity parameter evaluated in this study was clinical signs of toxicity. Animals (6/dose/timepoint) were euthanized at various timepoints after either oral administration (0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, and 72 hr) or intravenous administration (0.083, 0.25, 0.50, 0.75, 1, 2, 4, 8, 24, 48, and 72 hr) of thalidomide. In addition, three groups of animals (6/dose/timepoint) were euthanized at 4, 8 and 24 hours after 1% CMC administration and one group of animals (6/dose/timepoint) was euthanized at 2 hours after PEG-400 administration. A blood sample was collected from each of the euthanized animals for determination of plasma thalidomide levels. The plasma samples were diluted 1:1 with Sorenson's buffer to stabilize thalidomide during storage.

There were no clinical signs of toxicity observed for any of the animals in this study. Following oral administration of 200, 1000 or 2000 mg/kg thalidomide or 10 mg/kg intravenous thalidomide: 24% (16/66), 36% (24/66), 36% (24/66) and 1.5% (1/66) of the mice were observed with red or orange colored urine at the time of euthanasia, respectively.

The thalidomide concentrations (µg/ml) in mouse plasma following single oral dose administration of thalidomide in male mice is provided in the following table.

Dose (mg/kg)	0.5 hr	1 hr	1.5 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr	72 hr
200	22.17	16.90	17.50	13.47	10.47	7.17	4.02	1.36	0.00	0.00	0.00
1000	17.66	30.73	23.08	20.76	20.16	20.45	18.49	14.36	0.00	0.00	0.00
2000	19.56	27.31	25.58	19.32	28.97	18.47	19.21	12.78	0.00	0.00	0.00

The thalidomide concentrations (µg/ml) in mouse plasma following single intravenous dose administration of thalidomide in male mice is provided in the following table.

Dose (mg/kg)	0.083 hr	0.25 hr	0.50 hr	0.75 hr	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr	72 hr
10	5.13	1.18	1.57	0.79	0.68	0.00	0.00	0.00	0.00	0.00	0.00

The plasma pharmacokinetic parameters of thalidomide following single oral or intravenous dose administration of thalidomide in male mice is provided in the following table.

Dose (mg/kg)	Route	C_{max} (µg/ml)	T_{max} (hr)	$T_{½}e$ (hr)	AUC_{0-24hr} (µg*hr/ml)	F
200	PO	22.17	0.5	3.0	103	2.4
1000	PO	30.73	1	12.0	315	1.5
2000	PO	28.97	4	7.6	315	0.74
10	IV	5.13	0.083	0.38	2.12	1.0

Comments: Thalidomide elimination from plasma was monophasic following either single oral or intravenous thalidomide administration in mice. Thalidomide pharmacokinetics in mice following oral administration of suspended thalidomide in 1% CMC was non-linear. There was an apparent saturation of absorption which was evidenced by the leveling off of the AUC_{0-24hr} with increased oral thalidomide dose. The contract lab states that thalidomide has a maximum aqueous solubility of <0.1 mg/ml which may contribute to the relative constancy of C_{max} values following increasing oral dose administrations of thalidomide. This seems like a plausible explaination for the apparent plateau of the C_{max} values. This study did not report on the percentage of protein binding by thalidomide. This information would have been useful to evaluate the large discrepancy observed in several of the pharmacokinetic parameters calculated in this study.

The calculation of the F values in this study were not adequate because the F value should not be above one and the exact same dose levels need to be compared for a calculation of the F value. The F values calculated for the 200 and 1000 mg/kg oral dose groups were greater than unity. Three possible contributory factors were suggested by the contract lab for this finding. These factors were: 1) the AUC following the intravenous dose could be underestimated due to insufficiently frequent blood sampling; 2) exposure to the acidic enviroment of the stomach could result in less by hydrolysis for the oral route of adminstration as compared to the intravenous route; and 3) saturation of metabolism at concentrations above those observed following the intravenous dose. A direct comparison between the iv vs oral route may not be possible due to potential differences in metabolism (or formation of spontaneous hydrolysis products) between the different routes of administration. A potential difference in metabolite profile may affect the elimination of the parent compound thalidomide (which is the only species measured in this study). It would appear that the C_{max} via the iv route was not captured accurately potentially due to rapid degradation of thalidomide upon contact with neutral pH blood. In addition, the accuracy of the AUC values are questionable since the data was obtained from different mice at the various time points.

It is unclear as to which of these possiblities, or any of them, may explain the pharmacokinetics observed after iv and oral administration of thalidomide in mice in this study. Apparently, the design of this study was not adequate to capture the pharmacokinetic data accurately. However, it would appear that increasing the oral dose of thalidomide above 1000 mg/kg in the mouse will not generate any additional increase in systemic levels of thalidomide. For comparison purposes, administration of 200 mg/day and 400 mg/day of thalidomide to humans yields a AUC

value of ~18.9 ± 3.28 µg*hr/ml and ~36.4 ± 9.55 µg*hr/ml, respectively, based on the clincal pharmacokinetic data submitted by Celgene in this NDA. Therefore, the AUC value for the 1000 mg/kg dose in this study is ~8.5 times greater than that for the highest purposed clinical dose for ENL.

2. Analysis of thalidomide and metabolites in mouse plasma

Study Number: N002168B; Performing Organization: [ ] Date Study Completed: 12-10-96; Drug Lot Number: Thalidomide, Lot# 574-574-96-003; Animal Strain: male —:CD-1 mice (36 days for treated mice and 71 days for untreated mice); Number of Animals: 3 for thalidomide and 7 for untreated control; Dose: 3000 mg/kg/day; Route of Administration: oral (gavage); Vehicle - 1% CMC; Dose Volume: 10 ml/kg of a 300 mg/ml stock; Duration: 3 days; GLP Study: No.

The purpose of this study was to determine the level of thalidomide and potential hydroxylated metabolites in mouse plasma after oral administration of thalidomide. The treated mice were dosed with the test article once per day for three days. Three samples of mouse plasma (2, 4 and 6 hr postdose after the third day) were obtained by cardiac puncture for analysis. The plasma samples were diluted 1:1 with Sorenson's buffer to stabilize thalidomide during storage. Plasma samples were obtained from untreated control mice for comparison. The mouse plasma samples were analyzed by HPLC and the retention times of the peaks were compared to the retention times of four standards [N-hydroxythalidomide (N-OH-thal), 3-hydroxythalidomide (3-OH-thal), 4-hydroxythalidomide (4-OH-thal) and thalidomide].

The extracts of the mouse plasma from thalidomide treated mice contained four peaks that absorbed at 230 nm that were not seen in control plasma extracts. These peaks had retention times (RT) of 1.2, 1.8, 3.1, and 3.4 min. The first two peaks (RT = 1.2 and 1.8 min) did not have retention times that matched any of the standards and may represent metabolites (possibly hydrolysis products) other than the hydroxylated metabolites that were used as standards in this experiment. Another possibility for these two peaks is that they may represent blood components that were increased after thalidomide treatment. The third and fourth peak seen in the plasma samples derived from treated animals (RT = 3.1 and 3.5 min) matched the retention times for 4-OH-thal and thalidomide, respectively. The contract lab was not able to accurately quantitate the level of either peak in this experiment.

Comment: The results from this preliminary experiment suggest that 4-OH-thal is a potential metabolite formed in mice after thalidomide administration.

3. Pharmacokinetics and bioavailability of thalidomide following single oral or intravenous dose administration of thalidomide in male rats

Study Number: — 835-CEL-001-95; Performing Organization: [redacted]
Date Study Completed: 10-3-96; Drug Lot Number: Thalidomide, Lot# 574-574-95-001; Animal Strain: male Sprague-Dawley rat (7 - 8 weeks; 225 - 256 g); Number of Animals: 3/dose/timepoint; Dose: oral - 100, 500 or 1,000 mg/kg in 1% CMC, iv - 5 mg/kg in PEG-400; Route of Administration: oral (gavage) or intravenous; Vehicle - 1% CMC (oral) and PEG-400 (iv); Dose Volume: oral - 5 ml/kg of 20, 100 and 200 mg/ml stock, iv - 2 ml/kg of a 2.5 mg/ml stock; Duration: 72 hours; GLP Study: Yes.

The purpose of this study was to determine the plasma pharmacokinetics and bioavailability of thalidomide following single oral or intravenous dose administration of thalidomide in male rats. The only toxicity parameter evaluated in this study was clinical signs of toxicity. Animals (3/dose/timepoint) were euthanized at various timepoints after either oral administration (0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, and 72 hr) or intravenous administration (0.083, 0.25, 0.50, 0.75, 1, 2, 4, 8, 24, 48, and 72 hr) of thalidomide. In addition, three groups of animals (3/dose/timepoint) were euthanized at 4, 8 and 24 hours after 1% CMC administration and one group of animals (3/dose/timepoint) was euthanized at 1 hour after PEG-400 administration. A blood sample was collected from each of the euthanized animals for determination of plasma thalidomide levels.

There were no clinical signs of toxicity observed for any of the animals in this study. The following number of animals administered 5 mg/kg intravenous thalidomide in PEG-400 were observed with red urine at the time of euthanasia: 2/3 (0.25 hr), 3/3 (0.5 hr), 2/3 (0.75 hr), and 3/3 (1 hr).

The thalidomide concentrations (µg/ml) in rat plasma following single oral dose administration of thalidomide in male rats is provided in the following table.

Dose (mg/kg)	0.5 hr	1 hr	1.5 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr	72 hr
100	6.83	12.23	20.32	19.35	20.67	21.59	17.39	17.37	1.61	0.00	0.00
500	8.06	15.95	22.17	22.39	28.11	28.76	24.35	34.42	30.57	0.00	0.00
1000	10.55	16.60	21.35	20.81	28.24	29.90	26.95	25.87	23.81	0.00	0.00

The thalidomide concentrations (µg/ml) in rat plasma following single intravenous dose administration of thalidomide in male rats is provided in the following table.

Dose (mg/kg)	0.083 hr	0.25 hr	0.50 hr	0.75 hr	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr	72 hr
5	6.79	6.34	5.58	4.80	4.31	3.41	1.66	0.00	0.00	0.00	0.00

The plasma pharmacokinetic parameters of thalidomide following single oral or intravenous dose administration of thalidomide in male rats is provided in the following table.

Dose (mg/kg)	Route	C_{max} (µg/ml)	T_{max} (hr)	$T_{½}e$ (hr)	AUC_{0-48hr} (µg*hr/ml)	F
100	PO	21.60	6	3.5	348.5	0.98
500	PO	34.40	12	4.3	1063	0.60
1000	PO	29.90	6	4.6	882.9	0.25
5	IV	6.79	0.083	2.0	17.84	1.0

Comments: Thalidomide elimination from plasma was monophasic following either single oral or intravenous thalidomide administration in rats. Thalidomide pharmacokinetics in rats orally administered thalidomide were consistently non-linear. C_{max} (21.6, 34.4 and 29.9 µg/ml) and AUC_{0-48hr} (348.5, 1063, 882.9 µg*hr/ml) clearly plateaued for the oral doses of 100, 500 and 1,000 mg/kg, respectively. The low aqueous solubility of thalidomide may contribute to the constancy of C_{max} values. The oral half life (apparent $T_{½}e$) increased with dose (3.5, 4.3 and 4.6 hours for the 100, 500 and 1000 mg/kg dose groups, respectively). There is concern whether the calculation of the F values in this study were accurate, similar to what was stated for the mouse pharmacokinetic study discussed previously. The exact same dose levels need to be compared for an accurate calculation of the F value. Therefore, an accurate estimate of oral bioavailability can not be determined from this study. Elimination from plasma was rapid and monophasic following intravenous administration of thalidomide in rats. A direct comparison between the iv vs oral route may not be possible due to potential differences in metabolism (or formation of spontaneous hydrolysis products) between the different routes of administration. A potential difference in metabolite profile may affect the elimination of the parent compound thalidomide (which is the only species measured in this study). It would appear that the C_{max} via the iv route was not captured accurately potentially due to rapid degradation of thalidomide upon contact with neutral pH blood. In addition, the accuracy of the AUC values are questionable since the data was obtained from different rats at the various time points.

It would appear that increasing the oral dose of thalidomide above 500 mg/kg in the rat will not generate any additional increase in systemic levels of thalidomide. For comparison purposes

between AUC values obtained in this study and observed clinical AUC values, administration of 200 mg/day and 400 mg/day of thalidomide to humans yields a AUC value of ~18.9 ± 3.28 µg*hr/ml and ~36.4 ± 9.55 µg*hr/ml, respectively, based on the clincal pharmacokinetic data submitted by Celgene in this NDA. Therefore, the maximum AUC value for the 2 highest dose groups in this study is ~ 24.3 - 29.2 times greater than the highest proposed clinical dose for ENL.

4. Pharmacokinetics of thalidomide following daily oral dose administration of thalidomide for one week in beagle dogs

Study Number: — 832-CEL-002-95; Performing Organization: [ ] Date Study Completed: 10-4-96; Drug Lot Number: Thalidomide, Lot# 574-574-95-001; Animal Strain: Beagle dog (5 - 7 months; 7.97 - 9.63 kg); Number of Animals: 1/sex/group; Dose: oral - 12, 100, 500, 1,000 or 2,000 mg/kg thalidomide as a dry powder loaded into [ ] size #12 gelatin capsules, iv - 0.5 mg/kg in PEG-400; Route of Administration: oral or intravenous; Dose Volume: iv - 0.2 ml/kg of a 2.5 mg/ml stock via a 5 minute infusion into the cephalic vein; Duration: 7 days; GLP Study: Yes.

The purpose of this study was to determine the plasma pharmacokinetics of thalidomide following single daily dose adminstration for seven consecutive days, to determine the plasma pharmacokinetics of thalidomide following a single intravenous dose administration of thalidomide, and to estimate the absolute oral bioavailability of thalidomide in male Beagle dogs. [ ] conducted the in-life (dosing, sample collection, and sample shipment) aspects of the study. [ ] analytical laboratory conducted sample assay and forwarded audited sample assay results to — — performed the appropriate pharmacokinetic analysis of the sample assay results and summarized the in-life and analytical results and analysis in a report.

This study was a six treatment study in five groups of two Beagle dogs (1 male and 1 female) which were administered a daily oral dose of thalidomide (12, 100, 500, 1,000 or 2,000 mg/kg) in gelatin capsules for one week. Heparinized blood and derived plasma samples were collected serially (0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours) post dose administration on Days 1 and 7. Following a seven day washout period, two animals administered 2,000 mg/kg thalidomide orally were administered 0.5 mg/kg thalidomide by intravenous infusion and heparinized blood and derived plasma samples were collected serially (0.083, 0.167, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 32, and 48 hours) post intravenous dose administration. Pharmacokinetic parameters were determined from the dog plasma concentrations of thalidomide using standard noncompartmental pharmacokinetic methodology.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 7 in Beagle dogs are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (μg/ml)		T_{max} (hr)		$T_{½}$,e (hr)		$AUC_{0\text{-}24hr}$ (μg*hr/ml)	
		Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
12	M	2.26	2.56	1.5	1.5	1.4	1.4	7.05	7.55
12	F	2.92	2.92	2	1.5	2.2	1.5	11.2	8.37
100	M	3.92	8.68	2	4	2.4	2.2	20.7	58.7
100	F	5.18	9.00	1.5	2	2.9	2.2	23.4	57.1
500	M	18.8	20.6	2	4	2.8	3.1	116	201
500	F	8.82	10.1	12	2	5.3	3.0	103	60.4
1000	M	28.8	28.8	12	8	4.0	3.2	473	373
1000	F	11.9	25.2	12	1.5	2.8	3.6	170	261
2000	M	28.8	24.9	12	2	5.1	3.4	450	393
2000	F	30.4	21.5	6	4	2.7	3.1	350	205

Comments: Thalidomide absorption was dose proportional at low thalidomide dose levels and became non linear at higher dose levels following daily oral dose administration of thalidomide for one week in male and female dogs. The C_{max} and $AUC_{0\text{-}24hr}$ values plateaued in the 1,000 and 2,000 mg/kg dose groups. The non-linearity seen at higher dose may be a function of thalidomide's low water solubility. Thalidomide plasma $T_{½}$,e ranged from 1.4 to 5.3 hours and increased with increasing dose. There appeared to be no difference in the pharmacokinetics of thalidomide between males and females and between day 1 and day 7.

All of the plasma samples that were assayed after the intravenous dose were below the lower limit of quantitation. Therefore, intravenous plasma pharmacokinetics and absolute oral bioavailability could not be estimated in this study. It is interesting to note that the intravenous dose used in this dog study (0.5 mg/kg) was 10 times less than the dose used in rats (5 mg/kg) and 20 times less than the dose used in mice (10 mg/kg). This may have been a contributing factor to not being able to detect thalidomide in any of the plasma samples obtained after the intravenous dose.

It would appear that increasing the oral dose of thalidomide above 1000 mg/kg in the dog will not generate any additional increase in systemic levels of thalidomide. It is interesting to note that the maximum AUC levels obtained after oral dosing in dogs are at about the same levels as seen in mice. For comparison purposes between AUC values obtained in this study and observed clinical AUC values, administration of 200 mg/day and 400 mg/day of thalidomide to humans yields a AUC value of ~18.9 ± 3.28 µg*hr/ml and ~36.4 ± 9.55 µg*hr/ml, respectively, based on the clinical pharmacokinetic data submitted by Celgene in this NDA. Therefore, the maximum AUC value for

the highest dose group (~ 400 µg*hr/ml) in this study is ~ 11 times greater than the highest purposed clinical dose for ENL.

Nonclinical Toxicity and Safety Studies:

A) **Subchronic Toxicology Studies:**

1. 14 Day oral toxicity study of thalidomide in mice

Study Number: N002185A; Performing Organization: [ ] Date Study Completed: 10-18-96; Drug Lot Number: 574-574-96-003; Animal Strain: CD-1 mice (6 - 7 weeks; 20.3-32.4 g); Number of Animals: 10/sex/dose in main toxicity study (100 total), 3/sex/dose/timepoint (48/sex/dose; 384 total) in satellite pharmacokinetic study; Doses: 0, 50, 200, 750 and 3000 mg/kg; Route of Administration: Oral (gavage); Dose Volume: 10 ml/kg; Duration: 14 days; Vehicle Control: 1% aqueous carboxymethylcellulose; GLP Study: Yes.

The objective of the toxicology portion of this study was to establish doses for longer term toxicity studies and to assess and evaluate the toxicity characteristics of thalidomide when administered daily by oral gavage to mice for 14 days. The objective of the toxicokinetic portion of this study was to determine the pharmacokinetic behavior of thalidomide in plasma following the initial dose (Day 1) and after 8 days (Day 8) of dosing at levels of 50, 200, 750 and 3000 mg/kg in both male and female mice. The toxicokinetic profile during this study was obtained from a satellite group of animals that was run in parallel with the toxicity study.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, clinical pathology (hematology and serum chemistry), organ weights (adrenals, brain with brainstem, kidneys, liver, gonads {ovaries and testes}) and gross necropsy. Representative samples from all gross lesions and from a select group of organs and tissues (adrenals, brain with brainstem, heart, kidneys, liver, spleen and testes with epididymides) were preserved for histopathological evaluation.

Plasma samples were collected serially (0, 0.5, 1, 2, 4, 8, 12 and 24 hours) post dose administration on Days 1 and 8. Thalidomide plasma concentrations were analyzed by [ ] Pharmacokinetic parameters were determined by [ ] from the mouse plasma concentrations of thalidomide using standard noncompartmental pharmacokinetic methodology.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on body weight, food consumption, hematology or serum chemistries were noted in this study. Two animals in this study were noted to have hindlimb spray (one male in the 200 mg/kg dose group and one female in the 3,000 mg/kg dose group). This observation demonstrated no dose response relationship but was considered a treatment related effect. Treatment related effects were observed in the eyes of mice during the ophthalmic examination. Two males in the 50 mg/kg dose group had cataracts in both eyes at the end of the study. One female in the 3,000 mg/kg dose group had a cataract in the left eye. One male in the 3,000 mg/kg dose group was noted to have a cloudy cornea in the left eye. One female in the 50

mg/kg dose group and four in the 750 mg/kg dose group were noted to have corneal opacities. In addition, one female in the 200 mg/kg dose group had an irregular opening of the pupil of the left eye which the contract lab stated was most likely due to synechia.

A trend for increased liver weight in male mice (increase of ~13%) was observed in this study. This increase in liver weight in males was not statistically significant but may be biologically significant in light of the histopathological findings observed in the livers that will be discussed below. One gross observation was made during the necropsy. One high dose female was observed to have a dark, 7 x 6 x 5 mm mass involving the right kidney.

Histopathological examination of the organs and tissues obtained for this study revealed a microscopic change in the livers of thalidomide treated mice. Minimal centrilobular hepatocellular nuclear pleomorphism exhibited a dose dependent increase which was more pronounced in male mice. The incidence of this finding was: 1) 2/10 in the 200 mg/kg male mice, 5/10 in the 750 mg/kg male mice, 7/10 in the 3,000 mg/kg male mice and 2/10 in the 3,000 mg/kg female mice. This effect was characterized by an increase in enlarged and variably shaped hepatocellular nuclei primarily in the centrilobular portion of the hepatic lobule. A slight increase in cell size was evident in conjunction with the nuclear change. The contract lab stated that this cytomegaly and karyomegaly was noticeably more than the minimal variation in cell and nuclear size which is normally observed in the mouse liver. This finding correlated well with the trend (not statistically significant) for increased liver weights particularly in male mice. The renal neoplasm documented during the gross necropsy procedure was diagnosed as a hemangiosarcoma. The contract lab interpreted the renal neoplasm to be unrelated to treatment even though this type of tumor is considered to be an uncommon neoplasm in this mouse strain. Their rationale for this was that the large size of this neoplasm indicated that it must have been present for longer than two weeks and may even have been a congenital neoplasm. Based on this premise, I would concur with the contract's lab's classification of this neoplasm as an incidental finding.

There were several notations of reddened (discolored) urine which occurred across all dose groups. The appearance of discolored urine was noticed during the plasma sample gathering process. Most of the incidences of discolored urine occurred in male mice beginning with the one hour collection time point. Generally most of the observations were noted in the middle to high dose levels (750 to 3,000 mg/kg). More notations were made on study day 1 for the males from 1 to 4 hours post-treatment. There were no notations of discolored urine made after 4 hours post treatment in male mice. Only two females in the 3,000 mg/kg dose group had slightly reddened urine 2 hours post treatment on study day 1. Two of three females in the 3,000 mg/kg dose group were noted to have urine tinged pink at 8 hours post treatment. There were no notations of discolored urine in the female mice at 12 or 24 hours post treatment. More females had notations of discolored urine on study day 8 than on study day 1. There were six animals with this notation at one hour, four animals at two hours and six animals at four hours post treatment in the 200 mg/kg through 3,000 mg/kg dose groups. Fewer males were noted to have discolored urine on study day 8 (four at one hour, four at two hours and four at four hours post-administration in the 200 mg/kg through 3,000 mg/kg dose groups). There were no further notations of discoloration in either sex after four hours post treatment. The contact lab conducted an additional non-GLP study at the request of the sponsor to

determine whether the discoloration seen in the urine was due to blood. The details and results from this study are described below.

The contract lab conducted a non-GLP 7 day urinalysis study of thalidomide in CD-1 mice (study number - N002185C). Three groups of 5 mice/sex received daily oral gavage treatments of vehicle (1% carboxymethylcellulose) or thalidomide for seven days at daily dosages of 50 or 3,000 mg/kg. Any evidence of abnormal color was noted for any urine expressed spontaneously during handling and observation of each animal ~2 hours after treatment. Urine was collected overnight and examined visually for abnormal color. The urine samples (spontaneously expressed and 24 hour collections) were evaluated for blood by qualitative dipstick procedure. The mice were euthanized by carbon dioxide asphyxiation on Day 8. There was no necropsy performed on the mice in this study. The results from this study indicated that the discolored urine following thalidomide oral gavage to mice was not due to the presence of blood in the urine sample.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 8 in CD-1 mice are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml)		T_{max} (hr)		$T_{½}$,e (hr)		AUC_{0-24hr} (µg*hr/ml)	
		Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
50	M	2.60	3.32	0.73	0.45	0.52	0.90	6.53	5.61
50	F	4.21	3.79	0.50	0.43	1.29	0.31	8.71	7.85
200	M	6.63	9.54	1.06	0.50	1.49	3.46	29.81	43.64
200	F	9.50	10.07	1.41	1.04	1.71	1.60	61.58	41.09
750	M	14.10	15.91	1.68	1.79	2.45	1.35	88.12	112.71
750	F	20.36	15.29	2.63	2.00	1.87	4.46	182.34	80.67
3000	M	30.63	27.67	2.29	1.04	3.89	3.24	353.64	179.68
3000	F	37.59	28.18	3.31	3.39	2.35	1.53	572.64	191.31

Day 1 AUC values increased linearly with increased thalidomide dose. Day 1 C_{max} values did not demonstrate a linear increase with increased thalidomide dose. A plateau effect was seen for C_{max} in the highest dose group (3,000 mg/kg). Female mice demonstrated higher AUC and C_{max} values than male mice in each dose group for day 1 samples. Day 1 T_{max} and $T_{½}$,e values increased with increased thalidomide dose.

Day 8 AUC values did increase with increased dose except for the value at 3,000 mg/kg. The 3,000 mg/kg dose AUC value peak plasma concentration was not proportional to dose. Similarly, the increase in C_{max} with dose was not dose proportional. There was no longer a

difference seen between the male and female Day 8 AUC and C_{max} values which had been seen for the Day 1 values. Day 8 T_{max} and $T_{½}$,e values generally increased with increased dose.

These observations suggest that absorption processes are continuing for a longer period of time at the higher doses relative to the lower doses. In addition, there was a saturation of absorption at the highest dose with a blunting of the peak plasma concentration. The relative rate and extent of absorption amongst doses suggests little saturation of absorption at the lower doses in this study. Pharmacokinetic results from Day 1 and Day 8 are comparable. There was little evidence of accumulation which is consistent with the short $T_{½}$,e values.

Comments: The major toxicity elicited by thalidomide during this 14 day repeat dose study was an effect on the liver. Administration of the test article to CD-1 mice resulted in minimal centrilobular hepatocellular nuclear pleomorphism and an increase in cell size which was particularly expressed in male mice. This finding correlated well with the trend for increased liver weights in male mice. Treatment related effects were observed in the eyes of mice during the ophthalmic examination. The effects in the eyes did not show a dose-dependent relationship but were significant because with longer term treatment these effects may become more pronounced in the mice. Another significant observation in this study was the formation of discolored urine across all of the dose groups which did not show a dose dependent relationship. Results from an additional non-GLP study demonstrated that the discoloration in the urine was not due to blood. The results of this 14 day repeat dose oral toxicity study for thalidomide in CD-1 mice indicate that the NOTEL for the liver toxicity findings is 50 mg/kg in male mice and 750 mg/kg in female mice. The equivalent NOTEL for humans (based on an equivalent surface area dosage conversion) would be 4.2 mg/kg/day based on the male mice data (50 mg/kg/day ÷ 12 ≈ 4.2 mg/kg/day). For reference purposes, the highest proposed clinical dose of 400 mg/day would be equivalent to 5.7 mg/kg/day (400 mg/day ÷ 70 kg). If the doses are compared on an AUC basis, then the highest human dose of 400 mg/day yields an AUC ≈ 36.4 µg*hr/ml and would be ~4.5X higher than the NOTEL mouse dose of 50 mg/kg (AUC ≈ 8 µg*hr/ml) for liver toxicity. This could be a cause for concern clinically, but the effect on the liver may be a species specific effect because this type of hyperproliferative effect has been seen in mouse liver after other xenobiotic treatment regimens. It will be important to note if a similar effect is observed in the liver of other species treated with thalidomide. It is particularly noteworthy the appearance of cataracts in this 14 day study which is an unusual observation for this duration of treatment. Even though this effect did not demonstrate a dose-dependent response, it appeared to be related to thalidomide treatment. Therefore, careful analysis of the results from the 90 day repeat dose study in mice is warranted based on this observation.

Pharmacokinetic parameters from this study demonstrated that plasma thalidomide concentrations increase with dose except for the 3,000 mg/kg dose level. This is consistent with pharmacokinetic data obtained in previous studies which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. There was little evidence of accumulation which is consistent with the short $T_{½}$,e values. It is interesting to note that the pharmacokinetic parameters in this study are different than in the previously described PK study performed in mice discussed above. Part of the reason for this may be due to the low solubility of thalidomide which

could yield different suspension values in 1% CMC. This observation points out the importance of conducting concurrent PK studies with toxicity studies to obtain accurate toxicokinetic information for thalidomide. The maximum AUC value for the highest dose group (~ 450 µg*hr/ml) in this study is ~ 12 times greater than the highest proposed clinical dose for ENL (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml).

2. 13 Week oral toxicity study of thalidomide in mice (Note: Final report submitted on 6-4-97)

Study Number: N002185B; Performing Organization: [ ] Date Study Completed: 4-17-97; Drug Lot Number: 574-574-96-003; Animal Strain: CD-1 mice (6 - 7 weeks; 20.7-34.4 g); Number of Animals: 10/sex/dose in main toxicity study (80 total), 5/sex/dose in interim clinical pathology group (urine and blood samples collected on day 38) (40 total), 3/sex/dose/time point (48/sex/dose; 288 total) in satellite pharmacokinetic study; Doses: 0, 30, 300 and 3000 mg/kg; Route of Administration: Oral (gavage); Dose Volume: 10 ml/kg; Duration: 13 weeks; Vehicle Control: 1% aqueous carboxymethylcellulose; GLP Study: Yes.

The objective of the toxicology portion of this study was to assess and evaluate the toxicity characteristics of thalidomide when administered daily by oral gavage to mice for 13 weeks. The objective of the toxicokinetic portion of this study was to determine the pharmacokinetic behavior of thalidomide in plasma following the initial dose (Day 1) and after the final dose (Day 90) of dosing at levels of 30, 300 and 3000 mg/kg in both male and female mice. The toxicokinetic profile during this study was obtained from a satellite group of animals that was run in parallel with the toxicity study.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, clinical pathology (hematology and serum chemistry), urinalysis, organ weights (adrenals, brain with brainstem, kidneys, liver, thymus, epididymides and gonads {ovaries and testes}) and gross necropsy. Representative samples from all gross lesions and from a select group of organs and tissues [adrenals, aorta, bone marrow (femur), brain with brain stem (medulla/pons, cerebellar cortex and cerebral cortex), cecum, colon, duodenum, esophagus, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph node, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, spleen, stomach, testes (with epididymides), thymus, thyroids (including parathyroids), trachea, urinary bladder, and uterus] were preserved for histopathological evaluation. The following tissues were preserved and possible future examination will be performed if indicated by signs of toxicity or target organ involvement [cervical spinal cord, exoribital lacrimal glands, eyes (both with optic nerve), femur (including articular surface), lumbar spinal cord, mammary gland (female), salivary glands (mandibular), seminal vesicles, skin, mid-thoracic spinal cord, muscle (thigh), vagina and cervix].

Plasma samples were collected serially (0, 0.5, 1, 2, 4, 8, 12 and 24 hours) post dose administration on Days 1 and 91. Thalidomide plasma concentrations were analyzed by [ ]

] Pharmacokinetic parameters were determined by [ ] from the mouse plasma concentrations of thalidomide using standard noncompartmental pharmacokinetic methodology.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on body weight, food consumption or serum chemistries were noted in this study. The consistent clinical observation noted in this study was the formation of discolored urine. These notations of discolored urine were documented for the mice in the satellite group during obtaining blood for plasma samples. Discolored urine occurred across all treated dose groups in the males and in the mid- and high-dose groups in the females with no such observations in the control group. There was a definite increase in the number of notations of discolored urine as the dose level increased which suggested a dose related treatment effect. As discussed in the 14 day repeat toxicity study in mice above, the formation of discolored urine was not due to the presence of occult blood in the urine.

A dose dependent decreasing trend was seen in lymphocyte counts. This did not obtain statistical significance from the day 38 measurement but obtained statistical significance for the 300 mg/kg dose in male mice and the 3000 mg/kg dose in female mice from the day 92 measurement. These data may suggest a treatment induced decrease in circulating lymphocyte numbers after chronic treatment in mice.

Ophthalmic examinations demonstrated the following findings. At the end of the study one male in the 300 mg/kg dose group had a central lentricular opacity in the left eye. One male in the 3000 mg/kg group was noted to have a mild scleral hemorrhage in the lateral canthus region of the left eye. One female in the 300 mg/kg dose group was noted to have white retinas and that the vascular patterns on the retinas appeared obscure. Three females in the vehicle control group had corneal crystals. One female in the 30 mg/kg dose group, one in the 300 mg/kg dose group and two in the 3000 mg/kg dose group had notations of corneal crystals.

There was a significant increase in the liver weights in the 3000 mg/kg dose group for both male and female mice. There was also a significantly higher organ-to-weight ratio for livers in the 300- and 30000-mg/kg dose groups for both sexes. This increase in liver weight correlated with the histopathologic change noted (centrilobular hepatocellular hypertrophy) after administration of thalidomide in these CD-1 mice. This change was characterized by an increase in the size of the individual hepatocytes in the centrilobular areas resulting in tightly packed appearance and the elimination of any visible sinusoidal spaces. The cytoplasm of these hypertrophied hepatocytes had a finely granular consistency, and a smudgy eosinophilic tinctorial appearance. This lesion was observed in 10/10 male mice in all three dose groups. The severity of this change in the male mice showed a dose related increase. It was minimal for the low dose males, mild for the mid dose males and moderate for the high dose males. Mild centrilobular hepatocellular hypertrophy was observed in 10/10 high dose female mice. Centrilobular hepatocellular hypertrophy was not observed in the low and mid dose female mice. There were two neoplasms observed in this study. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. The contract lab concluded that since these were isolated lesions and since these are relatively common spontaneous neoplasms in CD-1 mice, both tumors were incidental and not related to the administration of the test article. I do not concur with this assessment. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are

relatively common spontaneous neoplasms in CD-1 mice, but I would anticipate that these tumors are relatively uncommon in CD-1 mice at this early of a time in their life span.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 90 in CD-1 mice are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml)		T_{max} (hr)		$T_{½},e$ (hr)		$AUC_{0\text{-}24hr}$ (µg*hr/ml)	
		Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
30	M	1.86	2.56	0.50	0.60	0.40	27.23	2.89	12.59
30	F	3.78	3.97	0.50	0.23	0.86	68.16	7.23	16.00
300	M	11.30	14.18	1.29	0.90	2.18	3.43	65.56	91.61
300	F	21.62	10.36	1.87	1.41	1.37	2.73	132.18	82.81
3000	M	31.26	27.72	1.00	1.50	ND	2.64	552.09	252.35
3000	F	46.11	16.00	3.00	1.91	2.55	2.48	647.66	172.81

ND - Not determined because not able to fit to curve due to unusual or insufficient data.

Day 1 AUC and C_{max} values increased with increased thalidomide dose. Female mice demonstrated higher AUC and C_{max} values than male mice in each dose group for day 1 samples. A general increase in Day 1 T_{max} and $T_{½},e$ values was observed with increased thalidomide dose. The high dose Day 1 $T_{½},e$ value was not able to be determined in this study. The contract lab suggests that this may be due to the limited data points with high variability. If this was indeed the case, then it suggests that further pharmacokinetic sampling should utilize more animals per time point.

Day 90 AUC and C_{max} values did increase with increased dose. However, the day 90 high dose AUC values had a definite plateau effect observed in this study. There was no longer the same trend in difference seen between the male and female Day 90 AUC and C_{max} values which had been seen for the Day 1 values. In many instances the female Day 90 AUC and C_{max} values were lower than the corresponding values for male animals. Results from this study suggest that absorption processes are continuing for a longer period of time at the higher doses relative to the lower doses. In addition, there was a saturation of absorption at the highest dose with a blunting of the peak plasma concentration especially evident after 90 days of repeat dose administration.

Day 90 T_{max} values generally increased with increased dose. However, the day 90 $T_{½},e$ values did not show an increase with increased thalidomide dose. The low dose day 90 $T_{½},e$ values were considerably greater that the mid and high dose group values. The contract's lab explanation for this result is that data points at the longest times for the two highest doses suggest that the $T_{½},e$ values for these doses may be substantially longer than the computer generated curve fit suggest.

If this assessment is correct, then this would suggest that the elimination rate is significantly increased under chronic repeat dose administration. This effect was more pronounced following 90 days of repeat dose administration than either 8 days (previous study) or one day of administration. The contract lab also states that there is a persistence of low levels of thalidomide after 90 days of repeat dose administration. These results may suggest that there is an increased potential for increased toxicity with chronic repeat dose administration in the mouse model.

Comments: The major toxicity elicited by thalidomide during this 90 day repeat dose study was an effect on the liver. Administration of the test article to CD-1 mice resulted in a dose dependent severity of centrilobular hepatocellular hypertrophy which was particularly expressed in male mice. This finding correlated well with the trend for increased liver weights in male mice. Induction of centrilobular hepatocellular hypertrophy after thalidomide administration was also observed in the 14 day repeat dose study in mice. Treatment related effects were observed in the eyes of mice during the ophthalmic examination. There was some concern raised when the results of the 14 day repeat dose study in mice demonstrated the formation of cataracts. This was an unusual observation, so particular attention was paid to effects on the eyes in this study. Unfortunately, the results from this study do not provide additional data on the risk of cataract formation after thalidomide treatment. In contrast, the results from this experiment highlighted another confusing and potentially treatment related effect on the eye for thalidomide. No notations of cataract formation were reported in this study. However, three females in the vehicle control group had corneal crystals and one female in each of the three dose group had notations of corneal crystals. The significance of this finding is unclear since there was a rather high incidence of corneal crystal formation observed in control animals. It is requested of the sponsor to submit additional information concerning the historical control data for CD-1 mice and the formation of corneal crystals. In addition, it would be helpful for the sponsor to provide a potential explanation for: 1) the formation of cataracts in the 14 day repeat dose toxicity study and not in the 90 day repeat dose toxicity study performed in mice and 2) the formation of corneal crystals in the 90 day repeat dose toxicity study and not in the 14 day repeat dose toxicity study performed in mice. Another possibly significant effect observed in this study was a dose dependent decreasing trend in lymphocyte counts. This effect did not obtain statistical significance until after day 90 of treatment and may suggest a potential toxicity of concern after chronic treatment with thalidomide.

Discolored urine was observed across all treated dose groups in the males and in the mid- and high-dose groups in the females with no such observations in the control group. There was a definite increase in the number of notations of discolored urine as the dose level increased which suggested a dose related treatment effect. This same effect was noted in the 14 day repeat dose study in mice discussed above. There were two neoplasms observed in this study. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. I do not concur with the contract lab's assessment that both tumors were incidental and not related to the administration of the test article since these were isolated lesions and are relatively common spontaneous neoplasms in CD-1 mice. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are relatively common spontaneous neoplasms in CD-1 mice, but I would anticipate that these tumors

are relatively uncommon in CD-1 mice at this early of a time in their life span. It is requested of the sponsor to submit additional information on the historical control data for CD-1 mice to validate the claim that the two types of tumors observed in this study are relatively common spontaneous neoplasms in CD-1 mice. In particular, special attention should be paid to clarifying at what time point in the life span of the CD-1 mice are alveolar-bronchiolar adenomas and uterine stromal polyps observed and what is their frequency level. One potential possibility for the presence of the alveolar-bronchiolar adenoma could be due to a murine virus infection of that particular animal. It is requested of the sponsor to provide additional information on the health status of the CD-1 mice used in this 13 week repeat dose toxicity study. Particular attention should be paid to the results of the 2 year carcinogenicity assay performed in mice that the sponsor will conduct as a phase IV commitment. The results from the 2 year carcinogenicity assay may help to determine the biological relevance of the two types of tumors observed in this 13 week repeat dose toxicity study.

The results of this 90 day repeat dose oral toxicity study for thalidomide in CD-1 mice indicate that the NOTEL for the liver toxicity findings is 300 mg/kg in female mice and could not be established for male mice since this effect was seen across all dose groups. This result could be a cause for concern clinically, but the effect on the liver may be a species specific effect because this type of hyperproliferative effect has been seen in mouse liver after other xenobiotic treatment regimens. It will be important to note if a similar effect is observed in the liver of other species treated with thalidomide.

Pharmacokinetic parameters from this study demonstrated that plasma thalidomide concentrations increased with increased dose with a definite plateau observed at the 3,000 mg/kg dose level. This is consistent with pharmacokinetic data obtained in previous studies which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. The results from this 90 day repeat dose study are more in line with the results from the 14 day repeat dose study discussed above then with the first phamacokinetic study described in mice. The 90 day $T_{½}$,e values calculated for this study gave the first indication of the possibility of potential accumulation of thalidomide after repeat dose administration. The low dose day 90 $T_{½}$,e values were considerably greater that the mid and high dose group values. The contract's lab explanation for this result is that data points at the longest times for the two highest doses suggest that the $T_{½}$,e values for these doses may be substantially longer than the computer generated curve fit suggest. If this assessment is correct, then this would suggest that the elimination rate is significantly increased under chronic repeat dose administration. This effect was more pronounced following 90 days of repeat dose administration than either 8 days (previous study) or one day of administration. The contract lab also states that there is a persistence of low levels of thalidomide after 90 days of repeat dose administration. These results may suggest that there is an increased potential for increased toxicity with chronic repeat dose administration in the mouse model. In addition to the possibility of parent thalidomide accumulation after repeat dose administration, there is the potential for accumulation of an active metabolite or spontaneous hydrolysis product after repeat dose administration. A separate study would need to be conducted which would measure the levels of metabolites and spontaneous hydrolysis products in addition to the parent compound thalidomide.

The maximum AUC value for the highest dose group (~ 650 µg*hr/ml) in this study is ~ 18 times greater than the highest proposed clinical dose for ENL (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml).

3. 14 Day range finding toxicity study of thalidomide in rats

Study Number: 2782-100; Performing Organization: [ ]
Date Study Completed: 11-21-96; Drug Lot Number: 470-470-95-502; Animal Strain: Fisher 344 rats (males: 157-192g; females: 117-139 g); Number of Animals: 10/sex/dose in main toxicity study (100 total), 3/sex/dose/timepoint (24/sex/dose; 192 total) in satellite pharmacokinetic study; Doses: 0, 50, 200, 750 and 3000 mg/kg; Route of Administration: Oral (gavage); Dose Volume: 10 ml/kg; Duration: 14 days; Vehicle Control: 1% aqueous carboxymethylcellulose; GLP Study: Yes.

The purpose of this study was to evaluate the toxicity of thalidomide when administered daily for 14 days by oral gavage to rats and to establish dose levels for a subsequent subchronic study. The objective of the toxicokinetic portion of this study was to determine the pharmacokinetic behavior of thalidomide in plasma following the initial dose (Day 1) and after 8 days (Day 8) of dosing at levels of 50, 200, 750 and 3000 mg/kg in both male and female rats. The toxicokinetic profile during this study was obtained from a satellite group of animals that was run in parallel with the toxicity study.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, clinical pathology (hematology and serum chemistry), organ weights (adrenals, brain with brainstem, kidneys, liver, testes with epididymides) and gross necropsy. Representative samples from all gross lesions and from a select group of organs and tissues (adrenals, heart, brain, kidneys, liver, spleen and testes with epididymides) were preserved for histopathological evaluation.

Plasma samples were collected serially (0, 0.5, 1, 4, 8, 12, 16 and 24 hours) post dose administration on Days 1 and 8. Thalidomide plasma concentrations were analyzed by [ ] Pharmacokinetic parameters were determined by [ ] from the rat plasma concentrations of thalidomide using standard noncompartmental pharmacokinetic methodology.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on clinical observations or on the eyes were observed in this study. Total body weight change was significantly decreased in all dose groups (except in the 50 mg/kg dose group) compared to control values. This effect was most pronounced in the high dose group. The total mean body weight gain of the high dose male rats was only 36% of the body weight gain of the control male rats. The total mean body weight gain of high dose female rats was only 42% of the body weight gain of control female rats. A significantly lowered mean food consumption value was noted for week 1 in the high dose male animals. Significant changes that may be related to treatment included lower mean values for platelet count in male animals in the 200 mg/kg dose group and all animals in the 750 mg/kg and 3,000 mg/kg dose groups. The decreases in platelet count were not dose dependent. Significantly lower mean values for total protein and albumin were observed in males in the 3,000 mg/kg dose group. This is probably a

treatment related effect since this change correlates with the lower weight gain observed in this dose group.

No treatment related gross pathology changes occurred in this study. The mean liver to body weight ratios were significantly greater for the high dose males and females, relative to controls. The contract lab's explanation for this is that these increases most likely reflect the lower terminal body weight values of the high dose group animals. This appears to be a reasonable assessment since there were no treatment related histomorphologic alterations noted in this study.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 8 in Fisher 344 rats are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml)		T_{max} (hr)		$T_{½}e$ (hr)		$AUC_{0\text{-}24hr}$ (µg*hr/ml)	
		Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
50	M	2.95	4.57	4.00	1.00	5.83	4.90	29.23	40.26
50	F	3.53	4.87	4.00	1.00	5.20	4.85	42.68	53.75
200	M	7.39	6.53	4.00	4.00	10.30	4.21	89.26	86.37
200	F	7.33	6.83	4.00	12.00	11.55	2.97	103.96	94.64
750	M	8.52	13.73	16.00	1.00	7.08	10.97	156.11	158.35
750	F	13.62	17.23	4.00	1.00	24.66	12.45	208.07	221.01
3000	M	23.57	15.47	16.00	8.00	8.86	13.99	385.29	253.61
3000	F	24.06	20.45	16.00	8.00	5.62	13.41	423.49	340.11

Day 1 AUC and C_{max} values increased with increasing dose but did not follow a linear response. Female rats demonstrated higher AUC values (but not statistically significant) than male rats in each dose group for day 1 samples.

Day 8 AUC and C_{max} values did increase with increased dose but did not follow a linear response. Female rats demonstrated higher AUC values (but not statistically significant) than male rats in each dose group for day 8 samples.

There was not as obvious a significant increase in $T_{½}e$ seen on either Day 1 or Day 8 with increased dose as was observed in the mice in the previous study described above. The AUC values at Day 8 were not significantly greater than the AUC values at Day 1. This result would indicate that there is not any significant carry over of thalidomide after repeat dosing. Even though the female rats tended to have a higher AUC value than male rats, this was not a statistically significant difference, which would indicate that no difference in the pharmacokinetics between male and female rats. The non-linear function of increase in AUC vs dose suggests a

saturation of the absorption process for thalidomide, especially at doses of 750 mg/kg/day and above.

Comments: The major toxicity elicited by thalidomide during this 14 day repeat dose toxicity study in rats was an effect on body weight which was most pronounced in the highest dose group in male and female animals. In addition, administration of the test article to F344 rats resulted in effects on platelet count and mean values for total protein and albumin. The results of this 14 day repeat dose oral toxicity study for thalidomide in F344 rats indicate that the NOTEL for effects on body weight is 50 mg/kg. The equivalent NOTEL for humans (based on an equivalent surface area dosage conversion) would be 7.1 mg/kg/day based on the male mice data (50 mg/kg/day ÷ 7 ≈ 7.1 mg/kg/day). For reference purposes, the highest proposed clinical dose of 400 mg/day would be equivalent to 5.7 mg/kg/day (400 mg/day ÷ 70 kg). If the doses are compared on an AUC basis, then the highest human dose of 400 mg/day yields an AUC ≈ 36.4 µg*hr/ml and would be approximately equivalent to the NOTEL rat dose of 50 mg/kg (AUC ≈ 40 µg*hr/ml) for effects on body weight gain. This could be a cause for concern clinically, but once again may be a species specific effect because this drastic effect on body weight gain was not seen in the previous 14 day repeat dose mouse study. It will be important to determine if this effect is potentiated in the 90 day repeat dose toxicity study in rats discussed below.

Pharmacokinetic parameters from this study demonstrated that plasma thalidomide concentrations increased with dose in a non-linear manner. This is consistent with pharmacokinetic data obtained in previous studies which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. There was little evidence of accumulation which is consistent with the Day 8 AUC values not being significantly greater than the Day 1 AUC values. It is interesting to note that the pharmacokinetic parameters in this study are different than in the previously described PK study performed in rats discussed above. Part of the reason for this may be due to the low solubility of thalidomide which could yield different suspension values in 1% CMC. This observation points out the importance of conducting concurrent PK studies with toxicity studies to obtain accurate toxicokinetic information for thalidomide. The maximum AUC value for the highest dose group (~ 425 µg*hr/ml) in this study is ~ 12 times greater than the highest purposed clinical dose for ENL (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml). It is interesting to note that the maximum AUC value in this study is more in line with all of the other maximum AUC values reported so far. Perhaps the maximum AUC reported in the single dose pharmacokinetic study in rats (maximum AUC ≈ 1,000 µg*hr/ml) was not an accurate value.

4) 13 Week oral toxicity study of thalidomide in rats with neurobehavioral assessments

Study Number: N002124A; Performing Organization: [redacted] Date Study Completed: 11-26-96; Drug Lot Number: 574-574-96-003; Animal Strain: Fisher 344 rats (~ 7 weeks old; males: 122 - 165 g; females: 116 - 135 g); Number of Animals: 10/sex/dose in main toxicity study (80 total), 10/sex/dose in the satellite pharmacokinetic group (60 total); Doses: 0, 30, 300, and 3000 mg/kg; Route of Administration: Oral (gavage); Dose Volume: 10 ml/kg; Duration:

13 weeks; Vehicle Control: 1% aqueous carboxymethylcellulose; GLP Study: Yes (draft final report).

The purpose of this study was to assess and evaluate the oral toxicity and the toxic characteristics of thalidomide when administered by oral gavage to rats for 13 weeks. The objective of the toxicokinetic portion of this study was to determine the pharmacokinetic behavior of thalidomide in plasma following the initial dose (Day 1) and after the final dose (Day 90) of dosing at levels of 30, 300 and 3000 mg/kg in both male and female rats. The toxicokinetic profile during this study was obtained from a satellite group of animals that was run in parallel with the toxicity study.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, neurobehavioral assessment including the functional observation battery (FOB) and motor activity, clinical pathology (hematology and serum chemistry; coagulation parameter including prothrombin time and activated partial thromboplastin time were measured at termination), thyroid function parameters, and urinalysis. There were two types of necropsies performed in this study. The tissues of the first 6 rats/sex/dose were fixed by whole body perfusion to facilitate histological evaluation of potential neuropathology, whereas tissues of the remaining 4 rats/sex/dose were preserved by traditional (immersion) measures for histopathological evaluation. Organ weights (liver, kidneys, adrenals, thymus and gonads) were collected on those animals that were fixed by immersion (4 rats/sex/dose). Representative samples from all gross lesions and from a select group of organs and tissues [adrenals, aorta, bone marrow (femur), brain with brain stem (medulla/pons, cerebellar cortex and cerebral cortes), cecum, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lungs, lumbar spinal cord, mammary gland (female), mesenteric lymph node, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, spleen, stomach, sural nerve, testes (with epididymides), thymus, thyroids (including parathroids), trachea, urinary bladder, and uterus] were preserved for histopathological evaluation. The following tissues were preserved and possible future examination will be performed if indicated by signs of toxicity or target organ involvement [cervical spinal cord, exoribital lacrimal glands, eyes (both with optic nerve), femur (including articular surface), salivary glands (mandibular), seminal vesicles, skin, mid-thoracic spinal cord, muscle (thigh), vagina and cervix].

Plasma samples were collected (0, 2, 8 and 18 hrs) post dose administration on Days 1 and 90. Plasma samples were obtained from 5 rats/sex/dose at 0 and 8 hours after Day 1 and D ay 90 and from 5 rats/sex/dose at 2 and 18 hours after Day 1 and Day 90. Thalidomide plasma concentrations were analyzed by [ ]. Pharmacokinetic parameters were determined by [ ] from the rat plasma concentrations of thalidomide using standard noncompartmental pharmacokinetic methodology.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. No treatment related effects on clinical observations, ophthalmic measurements or urinary parameters were observed in this study. There were thalidomide effects on body weight throughout the study at all dose levels and in both sexes. Low group mean body weights relative to vehicle control were evident within as few as eight days of thalidomide treatment

and persisted to some degree until termination at Day 91. The dose response effect of thalidomide on body weight was more consistent for male rats than for female rats. Male rats showed the following percent body weight decreases after 90 days of treatment: 8.2% (30 mg/kg), 11.3% (300 mg/kg) and 18.5% (3000 mg/kg). Female rats showed the following percent body weight decreases after 90 days of treatment: 6.0% (30 mg/kg), 7.0% (300 mg/kg) and 5.6% (3000 mg/kg). In addition, even though body weight was significantly reduced for female rats in all dose groups, the degree of effect on body weight was not as great in female rats as was observed in male rats. Male rats had a decreased food consumption in all dose groups but female rats showed no treatment related effect in food consumption.

There were five categories of behavioral patterns measured in the functional observational battery. The five categories were:

1) Autonomic (included measurements of defecation, urination, pupil responses, salivation, piloerection and lacrimation)
2) Muscle tone and equilibrium (included measurements of gait, gait score, mobility score, grip strength, landing footsplay, righting reflex)
3) Sensorimotor (included measurements of approach response, click response, touch response, tail pinch response, palpebral closure)
4) Central nervous system (included measurements of rearing arousal, posture, ease of removal, ease of handling, vocalizations, fur appearance, clonic convulsions, tonic convulsions)
5) Physiological (included measurements of body temperature, paw lick latency {thermal reactivity})

No treatment related effects were seen in the autonomic, central nervous system or physiological categories of measurements. In the muscle tone and equilibrium category, there was a treatment related effect on forelimb grip strength observed in male rats in the high dose group. Significant decreases in forelimb grip strength occurred in the high dose males at Week 4 (- 17.5 percent) and Week 8 (-12.6 percent) relative to control group males. A similar 12.5 percent decrease occurred at week 13, but was not significantly different from control. Hindlimb grip strength was not significantly altered in male animals and neither forelimb or hindlimb grip strength were affected in the females. The contract lab explained the decrease in forelimb grip strength in male rats as related to the body weight decreases evident in thalidomide treated male rats. I do not believe that the effect on forelimb strength seen in high dose male animals can be totally explained by a decrease in body weight. This may be a preliminary sign of peripheral neuropathy that is seen in humans after long term use of thalidomide. It will become important to follow this effect in longer term studies to see if the effect becomes more prevalent or becomes apparent at lower dose with increased time of exposure. There were no other treatment related effects in this category. One treatment related effect was observed in the sensorimotor category. Six out of ten male animals exhibited drooping eyelids (ptosis) or eyelids that were completely shut in the high dose group at week 13.

Motor activity measurements included individual animal total horizontal and vertical activities. Mean total horizontal activity did not differ between dose groups at any time period.

Vertical activity was significantly increased for the high dose male rats at week 4 and week 8 and for the high dose female rats at week 8. The significance of this finding is unclear since this effect was only seen at two timepoints in the high dose male rats and one timepoint in the high dose female rats.

Treatment related effects were seen in hematology parameters. The most noticeable effect was a dose dependent decrease in platelets seen in all dose groups at both the 6 and 13 week timepoints. This effect was more pronounced in male animals and their was ~25% decrease in the high dose animals at both timepoints. The contract lab stated that the decreased platelet values fell within historical values and are of questionable toxicological significance. However, I believe that since this decrease was statistically significant, showed a dose dependent response and was seen in the 14 day toxicity study described above, it is a significant effect. In male rats, decreased red blood cells and increased mean corpuscular volume and mean corpuscular hemoglobin might be indicative of mild anemia. No treatment related effects on coagulation values were observed in this study. Indications of minor leukopenia including lymphocytes, neutrophils, monocytes and eosinophils in treated males were also present. The treatment related significance of this may be questionable since there was no clear dose response observed and this effect was not seen consistently at both timepoint measurements.

A few effects on clinical chemistry parameters were observed in this study. Most of the effects did not demonstrate a clear cut dose-response trend so the biological relevance of these findings may not be significant. Serum glucose levels were decreased in some treated groups along with minor decreases in serum globulin and increased globulin/albumen ratios. Increased serum electrolyte levels (sodium and chloride) were reported after 6 weeks of treatment in male animals with accommodation after 13 weeks. A decrease in serum potassium levels was observed at both the 6 week and 13 week timepoints.

A treatment related effect on thyroid measurement parameters was observed in this study. A significant decrease in T3 levels was seen in all the female animal dose groups and in the mid dose male animal dose group. The effects on T3 levels were not dose dependent but showed an ~28% decrease in all female animal dose groups and an ~25% decrease in the mid dose male animal group. A dose dependent treatment related effect was seen in both total and free T4 levels in both male and female animals. The effect was more pronounced in female animals with the first significant decrease being observed in the low dose group for both the total and free T4 levels (~53% decrease in total T4 and ~54% decrease in free T4 levels in the high dose group). In male animals, significant decreases were observed in the mid and high dose groups (~37% decrease in total T4 and ~44% decrease in free T4 levels in the high dose group).

Thymus weight decreased in all treated groups relative to the analogous vehicle control levels. A possible dose response relationship was more consistent in the male rats than in female rats. Thymus weights were statistically significantly decreased relative to vehicle control in both the male and female animals in the mid dose group and in the male animals in the high dose group. No other treatment related effects on organ weights was observed in this study.

No treatment related histopathological effects were seen in this study. The results of the histopathological examination of the sural nerves showed no difference between the control and treated groups for either male or female animals. It is interesting to note that minimal axonal

degeneration of sural nerve fibers was observed in many of the male rats in this study. The occurrence of this was 8/10, 6/10, 6/10, and 6/10 for control, low, mid and high dose group male animals, respectively. The occurrence of this was 1/10, 0/10, 1/10, and 1/10 for control, low, mid and high dose group female animals, respectively. The contract lab states that the histomorphology of this lesion involving the sural nerve fibers is consistent with the early manifestation and diagnosis of radiculoneuropathy as it occurs in rats. Radiculoneuropathy is a common spontaneous degenerative lesion of the spinal cord and peripheral nerves which may result in posterior paralysis. Since this condition appears to be quite prevalent in male rats, it is questionable whether thalidomide treatment effects on the sural nerve can be determined in this species. It now becomes even more important to determine the results of this in the second species (dog) which the sponsor has submitted the 6 month results of a 1 year study in the current submission. The sponsor states that assessment of distal portions of the lumbar spinal cord are in progress.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Days 1 and 90 in Fisher 344 rats are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (µg/ml)		T_{max} (hr)		$T_{1/2}e$ (hr)		AUC_{0-18hr} (µg*hr/ml)	
		Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
30	M	7.47	7.68	2.36	2.00	1.60	4.06	45.35	57.82
30	F	10.40	10.78	2.28	3.03	1.84	2.75	63.99	91.04
300	M	21.80	14.94	3.81	3.84	4.61	11.49	289.34	197.92
300	F	31.07	20.56	3.72	3.49	11.75	9.86	347.22	280.62
3000	M	40.19	19.84	5.25	1.14	19.00	43.78	537.10	322.52
3000	F	41.53	36.00	18.00	3.96	ND	8.75	681.66	483.34

ND - Not Determined, not able to determine from the data.

Day 1 and Day 90 AUC and C_{max} values increased with increasing dose but did not follow a linear response. Female rats demonstrated higher AUC (but not statistically significant) than male rats in each dose group for day 1 samples. At a dose of 300 mg/kg, the observed Day 1 AUC_{0-18hr} is 64% of that expected for males and 54% of that expected for females (based on extrapolation of the 30 mg/kg AUC_{0-18hr} value). The observed Day 1 AUC's are 12% for males and 11% for females of those expected at the 3,000 mg/kg dose. At a dose of 300 mg/kg, the observed Day 90 AUC_{0-18hr} is 34% of that expected for males and 31% of that expected for females. The observed Day 90 AUC's are 6% for males and 5% for females of those expected at the 3,000 mg/kg dose. These observations suggest a saturation of absorption processes at these doses. This is further verified by a lack of proportional increases of C_{max} with dose as doses

increase. These observations also suggest that the absorption processes although saturated are continuing for a longer period of time at the higher doses relative to the lowest dose. Half-lives for the lowest dose are consistently lower than for the higher doses.

At the 30 mg/kg dose there is no carry over of thalidomide to the next day. At the 300 and 3,000 mg/kg doses there is a measurable concentration of thalidomide in the plasma. However, even with this carryover, no clear consistent increases in the AUC values was observed for the day 90 values compared to the day 1 values. This result would suggest that there is no potential for increased accumulation of thalidomide with repeat dosing. One possible explanation for this could be due to the rapid rate of hydrolysis of thalidomide that may serve as an extra elimination pathway to prevent thalidomide accumulation after repeat dosing.

Comments: A dose dependent decrease in body weight was observed in male rats. The treatment related effect on body weight was not as pronounced in female rats. Administration of the test article to F344 rats resulted in a dose dependent decrease in platelet count and mild leukopenia. A treatment related effect on thyroid function was observed in this study. Thymus weight decreased in all treated groups relative to the analogous vehicle control levels. The results of this 90 day repeat dose oral toxicity study for thalidomide in F344 rats indicate that the 30 mg/kg dose generated slight toxic effects in many of the significant effects noted in this study. Therefore, there is no clear cut NOTEL in this study.

Pharmacokinetic parameters from this study demonstrated that plasma thalidomide concentrations increased with dose in a non-linear manner. This is consistent with pharmacokinetic data obtained in previous studies which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. There was little evidence of accumulation which is consistent with the Day 90 AUC values not being significantly greater than the Day 1 AUC values. It is interesting to note that the pharmacokinetic parameters in this study are different than in the previously described PK study performed in rats discussed above. Part of the reason for this may be due to the low solubility of thalidomide which could yield different suspension values in 1% CMC. This observation points out the importance of conducting concurrent PK studies with toxicity studies to obtain accurate toxicokinetic information for thalidomide. The maximum AUC value for the highest dose group (~ 681 µg*hr/ml) in this study is ~ 19 times greater than the highest purposed clinical dose for ENL (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml).

5) 28 Day oral range finding toxicity study of thalidomide in beagle dogs

Study Number: 95542; Performing Organization: [ ] Date Study Completed: 2-16-96; Drug Lot Number: Thalidomide, Lot# 574-574-95-001; Animal Strain: Beagle dog (5 months; 7.97 - 9.63 kg); Number of Animals: 1/sex/group (10 total); Dose: 12, 100, 1,000 or 2,000 mg/kg thalidomide as a dry powder loaded into gelatin capsules; Route of Administration: oral; vehicle control - empty gelatin capsules; Duration: 28 days; GLP Study: Yes.

The purpose of this study was to determine appropriate doses for a chronic toxicity study of thalidomide in Beagle dogs. Thalidomide was administered daily by capsule to Beagle dogs for 28 days. The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, clinical pathology (hematology and serum chemistry), urinalysis, organ weights (kidneys, liver, ovaries, lungs, brain, testes and heart) and gross necropsy. Representative samples from all gross lesions and from a select group of organs and tissues (brain, skeletal muscle, testes, liver, ovaries, sciatic nerve, heart and aorta, kidneys and lungs) were preserved for histopathological evaluation. Minimal toxicokinetic analysis was performed in this study. Plasma samples were obtained in the following manner: 1) from one control animal and from the 12 mg/kg and 100 mg/kg dose groups following 26 days of dosing at 2 hours post dose and 2) from one control animal and from the 1,000 and 2,000 mg/kg dose groups following 27 days of dosing at 8 hours post dose. No pharmacokinetic parameters were calculated in this study and only the plasma levels of thalidomide reported from [ ]1 (the contract lab that performed the analysis) were included for this study.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. The primary clinical signs noted during the study period were discolored urine (green, brown) and evidence of what appeared to be unchanged test material in the feces of the thalidomide dosed dogs. The incidence (total number of days noted) of these findings are presented in the table below.

Animal Number	Dose (mg/kg/day)	Days	
		Discolored Urine	Fecal Test Compound
1	0	0	0
2	0	0	0
3	12	2	0
4	12	6	0
5	100	24	0
6	100	24	0
7	1000	29	19
8	1000	29	14
9	2000	28	28
10	2000	28	25

No treatment related effects were observed on body weights, food consumption, hematology, or organ weights. The only clinical pathology effect observed after treatment with thalidomide was a 14 - 30% decrease in serum glucose levels which did not demonstrate a dose response relationship. The organ weights of the control and the thalidomide dosed dogs were within normal limits. No treatment related effects on either gross or microscopic pathology were observed in this study.

The plasma levels of thalidomide are listed in the table below.

Animal Number	Dose (mg/kg/day)	Sampling Time	Plasma Concentration (μg/ml)
1	0	26 days - 2 hours post dose	0
2	0	27 days - 8 hours post dose	0
3	12	26 days - 2 hours post dose	0.69
4	12	26 days - 2 hours post dose	1.05
5	100	26 days - 2 hours post dose	1.44
6	100	26 days - 2 hours post dose	1.97
7	1000	27 days - 8 hours post dose	0.78
8	1000	27 days - 8 hours post dose	1.25
9	2000	27 days - 8 hours post dose	2.24
10	2000	27 days - 8 hours post dose	1.29

Comments: Thalidomide treatment did not cause any overt expression of toxicity in Beagle dogs at daily doses up to 2,000 mg/kg for 28 days in this study. Apparently Beagle dogs are much less sensitive to thalidomide induced toxicity than are rodents. The most striking observation in this study was the appearance of discolored urine (green and brown). Discolored urine (pink, orange or red) was also observed in the mouse 14 day repeat dose toxicity study, mostly at the 750 and 3000 mg/kg/day dose groups. A separate non-GLP study determined that the discolored urine was not due to the presence of blood in the urine. The presence of discolored urine in the mouse and dog study may indicate the metabolic formation of a chromaphore from thalidomide that is excreted in the urine. Red and blue substances have been reported from simple hydrolysis of thalidomide (Schumacher, H. Smith, R.L., and Williams, R.T. 1965a. The metabolism of thalidomide: The spontaneous hydrolysis of thalidomide in solution Brit. J. Pharmacol. 25: 324-337) as well as excreted in the urine of laboratory animals treated with thalidomide (Schumacher, H., Smith, R.L., and Wiliams, R.T. 1965b. The metabolism of thalidomide: The fate of

thalidomide and some of its hydrolysis products in various species. Brit. J. Pharmacol. 25: 338-351).

The pharmacokinetic data obtained in this study is of little value since only single timepoint samples were obtained for each animal. Therefore, neither C_{max} or AUC values could be calculated to make a comparison with the 7 day pharmacokinetic study conducted in dogs which is described in the pharmacokinetic section above. The only benefit that obtaining this data provides is to indicate that there were measurable levels of thalidomide obtained in this 28 day repeat dose toxicity study in Beagle dogs.

6) A 52 week oral toxicity study of thalidomide in beagle dogs

Study Number: 96583; Performing Organization: [ ] Date Interim Report Completed: 12-11-96; Drug Lot Number: Thalidomide, Lot# 574-574-95-001, 574-574-95-002 and 574-574-95-003; Animal Strain: Beagle dog (8-10 months); Number of Animals: 8/sex/group for control and high dose groups, 6/sex/group for low and mid dose groups (56 total); Dose: 43, 200 or 1,000 mg/kg thalidomide as a dry powder loaded into [ ] size 11 gelatin capsules; Route of Administration: oral; vehicle control - empty [ ] size 11 gelatin capsules; Duration: 52 weeks; GLP Study: Yes (6 month interim report).

The purpose of this study was to assess the systemic toxicity of thalidomide given as single daily oral doses in capsules to dogs for a period of twelve months. This study was divided into three phases: 1) an interim sacrifice at six months of 2 dogs/sex/group; 2) a terminal sacrifice at 12 months of 4 dogs/sex/group; and 3) a sacrifice after a one month recovery period of 2 dogs/sex/group in the control and high dose groups. The results of the 6 month interim sacrifice are presented in this report.

The dose range for the 1 year dog study was selected on the basis of the results of the 1 week pharmacokinetic study in the dog. This study indicated that repeated administration of thalidomide *via* capsule produced increasing drug exposure in response to increasing doses through 1000 mg/kg/day, but no further increase in exposure at 2000 mg/kg/day, probably due to limitations in absorption. Hence, 1000 mg/kg/day was selected as the highest dose in the 1 year dog study. From extrapolation of plasma data from the lowest doses in the dog study and published data measuring plasma levels of thalidomide in healthy male volunteers (Chen, T.L., Vogelsang, G.B., Petty, B.G., Brundrett, R.B., Noe, D.A., Santos, G.W., and Colvin, O.M. 1989. Plasma pharmacokinetics and urinary excretion of thalidomide after oral dosing in healthy male volunteers. Drug Metabol. Dispos. 17: 402-405), it was estimated that a daily dose of 43 mg/kg would produce a similar exposure of thalidomide in the dog as a human receiving a single daily oral dose of 400 mg. The 200 mg/kg dose was selected as a logarithmic midpoint between the 43 mg/kg and 1000 mg/kg dose levels.

The toxicity parameters that were evaluated in this study included mortality/morbidity, clinical signs, body weights, food consumption, ophthalmic exam, physical examinations (including rectal temperature, respiration rate and electrocardiography), neurological examination (including cerebral, cerebellar, cranial nerves, proprioception, posture and gait, reflexes and sensation), nerve

conduction, clinical pathology (hematology and serum chemistry), urinalysis, thyroid function (analysis of TSH, T3 and T4 levels), endocrine function (analysis of prolactin, estradiol, cortisol, corticosterone, aldosterone and ACTH levels). A gross necropsy was performed with measurement of organ weights (brain, heart, liver, thymus, kidneys, adrenal glands, gonads, thyroid, epididymides, spleen and uterus). Representative samples from all gross lesions and from a full spectrum of organs and tissues (including sciatic and sural nerves) were preserved for histopathological evaluation.

Toxicokinetic analysis was performed in this study. Duplicate blood samples were collected from all dogs by jugular venipuncture on Day 1 and Day 178 (week 26) at 0, 1, 2, 4, 6, 8 and 24 hours post dose administration. Plasma samples were processed according to Eriksson, et. al., 1992. Samples collected on Day 1 were analyzed for drug levels by [ ]. Samples collected on Day 178 were sent to Celgene for analysis of drug levels. The results of these analyses were presented in this report.

No treatment related deaths were observed in this study. No animals were sacrificed moribund in this study. The primary clinical signs noted during the study period were green colored urine, unchanged test article in the feces and enlarged mammary tissue. Green urine was intermittently observed for 10/12 low dose animals, and all of the mid and high dose animals. The time of onset of discolored urine was governed by dose with the high dose animals first producing green urine at an earlier point during the study (as early as Day 1) than the low and mid dose animals (as late as Day 91). What appeared to be unchanged test article was intermittently observed as a white residue in the feces of 9/12 dogs in the low dose group and all the mid and high dose animals. The presence of test article in the feces was observed with greater frequency with increasing dose.

Slight to moderate enlargement of the mammary tissue was observed in two low dose and one high dose female dogs. The enlarged mammary tissue of one low dose female appeared light blue in color. A white watery substance, presumed to be milk, could be expressed from the nipples of the affected high dose female. No microscopic changes were seen in the mammary tissue of the dogs necropsied at the interim sacrifice.

No treatment related effects were seen on body weights, food consumption, ophthalmic exam, physical examinations, neurological examination, nerve conduction, clinical pathology, urinalysis, thyroid function, endocrine function or organ weights. Some dogs had gross pathological findings observed for the bones, especially the cranial bones. Two of four mid dose dogs and three of four high dose dogs had discoloration (green and/or yellow) of the cranial bones. In addition, one of the affected high dose animals exhibited similar discoloration of the rib, femur and orbit. These findings suggested a test article related effect and a dose effect trend. It is interesting to note that although these findings were present during necropsy, subsequent observation of bones in fixative revealed that the discoloration had become less pronounced in the bones. This finding suggested that possible leaching or breakdown of the agent(s) responsible for discoloration of the affected bones had occurred in the fixative. These macroscopic observations of affected bones did not have microscopically correlating lesions. There were no test article related effects seen microscopically with the exception of sural nerve tissue in the high dose animals. Morphological and pathological evaluation of ultrasections of the sural nerve tissue suggested a very slight dilation of the axon cylinder with loss of neurofilaments in the high dose male dogs. No other

effects on sural nerves were observed in other treated animals. The contract lab stated that these changes may be associated with processing rather than thalidomide-induced alterations of the axoplasm. It will remain to be seen whether this effect was treatment related or not based upon the results of the 1 year treated animals.

The plasma pharmacokinetic parameters of thalidomide following oral administration on Day 1 in Beagle dogs are summarized in the following table. This report only contains the pharmacokinetic results after Day 1 dose administration. The Week 26 pharmacokinetic parameters are not presented in this report. Presumably the week 26 results will be presented with the final 1 year study report.

Dose (mg/kg)	Sex	C_{max} (μg/ml)	T_{max} (hr)	$T_{½}$,e (hr)	$AUC_{0\text{-}24hr}$ (μg*hr/ml)
43	M	2.03	2.83	2.71	14.74
43	F	2.43	1.83	3.63	7.82
200	M	2.56	3.00	3.89	26.62
200	F	4.99	5.50	1.97	49.22
1000	M	7.92	6.75	21.67	104.49
1000	F	8.57	11.75	7.28	112.10

The results of the day 1 pharmacokinetic parameters indicate that at the 43 mg/kg dose in either sex, thalidomide rises to maximum concentrations in the plasma of around 2 μg/ml at 2 to 3 hours and is cleared with a $T_{½}$,e of about 3 hours producing an $AUC_{0\text{-}24hr}$ between 8 and 15 μg*hr/ml. At the 200 mg/kg dose in either sex, thalidomide rises to maximum concentrations in the plasma of around 3 to 5 μg/ml at about 3 to 5.5 hours and is cleared with a $T_{½}$,e of about 2 to 4 hours producing an $AUC_{0\text{-}24hr}$ between 27 and 49 μg*hr/ml. At the 1000 mg/kg dose in either sex, thalidomide rises to maximum concentrations in the plasma of around 8 to 9 μg/ml at about 7 to 12 hours and is cleared with a $T_{½}$,e of about 7 to 22 hours producing an $AUC_{0\text{-}24hr}$ between 104 and 112 μg*hr/ml.

Concentrations of plasma thalidomide increase with increased dose, but not in a linear fashion, which suggests some degree of saturation of the absorption of thalidomide at the highest dose. The relative AUC's with increased dose suggests a partial saturation of absorption at the highest dose as well. As the dose increases, thalidomide concentrations are present in the plasma for a longer period of time. There were no clear differences observed in this study between female and male dogs with respect to plasma pharmacokinetics of thalidomide. It is important to note that the coefficients of variation in all the pharmacokinetic parameters determined amongst animals treated identically in this study were quite high which indicated that substantial variation in the handling of thalidomide was seen in individual animals. This was probably due to the low aqueous solubility of thalidomide. Another possibility for the variability in the pharmacokinetic

parameters could be related to the fed vs fasted status of the dogs prior to thalidomide treatment. In dogs, the pH in the stomach varies according to how recently the dogs have eaten (i.e. ~pH of 5 in fasted dogs and ~pH of 2 in fed dogs). The difference in pH for the fed vs fasted state would have a dramatic effect on the rate of spontaneous hydrolysis of thalidomide and in turn on the pharmacokinetic measurements in this nonclinical study.

Comments: Thalidomide treatment did not cause any overt expression of toxicity in Beagle dogs at daily doses up to 1,000 mg/kg for 6 months in this study. The appearance of discolored urine (yellow and/or green) was noted again in this study and demonstrated a dose related increase in prevalence. The presence of test article in the feces was observed with greater frequency with increasing dose suggesting that not all of the administered dose was absorbed in the higher dose groups. There appeared to be a treatment related effect on enlargement of mammary tissue. It will be interesting to see if this effect persists in the 1 year final report for this study. A dose dependent increase in discoloration of the cranial bones was observed in this study. There were no microscopic correlation observed for the macroscopic effects observed on the mammary tissue and cranial bones. Morphometry and pathologic evaluation of ultrasections did identify a slight axonal swelling with loss of neurofilaments which could not be entirely ruled out as treatment related in two high dose male dogs. The final results after completion of one year of repeat dosing in dogs will help determine if this could be an indicator in an animal model to predict the thalidomide associated neuropathy that has been observed in humans after treatment with thalidomide.

Pharmacokinetic parameters from this study demonstrated that plasma thalidomide concentrations increased with dose in a non-linear manner. This is consistent with pharmacokinetic data obtained in previous studies which demonstrated a saturation of the absorption of thalidomide at the highest dose levels. Only the day 1 pharmacokinetic parameters were presented in this 6 month interim report. It is hoped that the sponsor will present the results from the 6 month and final 1 year sampling in the final report for this 1 year toxicity study in dogs. The maximum AUC value for the highest dose group (~ 112 µg*hr/ml) in this study is ~ 3 times greater than the highest purposed clinical dose for ENL (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml).

It is important to note that the maximum AUC obtained in this study (~ 100 µg*hr/ml) is substantially lower (~ ¼X) than was obtained in the 7 day repeat dose pharmacokinetic study (~400 µg*hr/ml) described above. The AUC value for the 43 mg/kg/day group (~ 10 µg*hr/ml) was ~ ½X that observed clinically after a 200 mg/day dose of thalidomide (~19 µg*hr/ml). This raises some concern over whether the doses used in this 1 year study will be high enough to accurately characterize the chronic toxicity profile for thalidomide in dogs. It will be important to see the pharmacokinetic results from the 6 month and 1 year time points for a more accurate assessment of the adequacy of the dose range used in this 1 year dog study. The role of fed vs fasted status may play a factor in the variability of the AUC values. It will be requested of the sponsor in the recommendations section to clarify the fed vs fasted status prior to thalidomide treatment in this study.

B) Genetic Toxicology Studies:

1. *Ames/Salmonella-E. coli* reverse mutation assay on thalidomide

Study Number: — 301-CEL-001-95; Performing Organization: [ ]
Date Study Completed: 11-7-95; Drug Lot Number: 574-574-95-001; Tested in: *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of exogenous metabolic activation (microsomal S9 fraction); Concentration: Thalidomide was tested at 50, 167, 500, 1670, 5000 and 10,000 µg/plate (thalidomide precipitated from solution at doses ≥ 5000 µg/plate) . Appropriate positive control mutagens were run for each tester strain; Vehicle control: DMSO; GLP Study: Yes.

Under the conditions of this study, the test substance was not mutagenic in the Ames test, with or without metabolic activation, at concentrations ≤ 10 mg/plate.

Comments: A rapid rate of spontaneous hydrolysis for thalidomide would be expected in the neutral pH of the media for this assay. Therefore, there is some concern about the stability of thalidomide under the conditions of this *in vitro* study and the potential validity of the results from this test to assess potential genetic toxicology of thalidomide.

2. AS52/XPRT mammalian cell forward gene mutation assay on thalidomide

Study Number: — 314-CEL-001-95; Performing Organization: [ ]
Date Study Completed: 6-6-96; Drug Lot Number: 574-574-95-001; Tested in: AS52 Chinese hamster ovary cells in the presence and absence of exogenous metabolic activation (microsomal S9 fraction); Concentration: Thalidomide was tested at 0.500, 1.67, 5.00, 16.7, 50.0, 167, 500, and 1000 µg/ml in the non-activated system and at 0.0167, 0.0500, 0.167, 0.500, 1.00, 1.67, 2.50, 5.00, 10.0, 16.7, 25.0, 50.0, 100, 167, 250, 500, and 1000 µg/ml in the activated system (exposed to thalidomide for 5 hr). A confirmatory study was run at thalidomide concentrations of 1.00, 5.00, 10.0, 25.0, 50.0, 100, 250, 500 and 1000 µg/ml with and without S9. Appropriate positive control compounds were run for each assay condition; GLP Study: Yes.

Under the conditions of this study, the test substance was not clastogenic in the AS52/XPRT Mammalian Cell Forward Gene Mutation assay, with or without metabolic activation, at concentrations ≤ 1000 µg/ml.

Comments: The comments that were stated for the previous *in vitro* genetic toxicology study are valid for this study as well. It may be advisable to conduct an *in vivo* mammalian cell gene mutation assay for thalidomide to generate additional data concerning the potential genetic toxicology for thalidomide.

3. *In vivo* micronucleus test with thalidomide in mouse bone marrow erythropoietic cells

Study Number: 309-CEL-001-95; Performing Organization: [redacted]
Date Study Completed: 3-19-96; Drug Lot Number: 574-574-95-001; Animal Strain: CD-1 mice (30-39 g for males and 22-24 g for females); Number of Animals: 5/sex/group; Dose: 500, 2500 and 5000 mg/kg; Route of Administration: intraperitoneal; Dose Volume: 20 ml/kg; Vehicle control: 1% Carboxymethylcellulose; Positive control: cyclophosphamide (60 mg/kg); Duration: 72 hours; GLP Study: Yes.

The number of micronucleated polychromatic erythrocytes in mouse bone marrow was determined 24 hours (test article, vehicle control and positive control), 48 hours (test article and vehicle control) and 72 hours (test article and vehicle control) after treatment as a measure of clastogenic potential. The ratio of polychromatic to normochromatic erythrocytes per 1000 erythrocytes was also determined at the same time points as a measure of toxicity. There was no indication of toxicity expressed over the dose range tested in this study.

Under the conditions of this assay, thalidomide did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes at a dose ≤ 5000 mg/kg in CD-1 mice and was considered negative in the mouse bone marrow micronucleus assay.

Index of Nonclinical Protocols:

This submission contains nonclinical protocols for the following studies:

1) 104 week mouse oncogenicity
2) 104 week rat oncogenicity
3) Reproductive toxicity dose range finding study in male rabbits
4) Reproductive toxicity dose range finding study in female rabbits
5) Segment I reproductive toxicity study in rabbit
6) Segment III reproductive toxicity study in rabbit

Nonclinical Protocols:

1) 104 week mouse oncogenicity

Title: Oncogenicity Study of Thalidomide in Mice
Protocol number: 7089
Performing organization: [redacted]
Species/Strain: – CD-1 mice (6-8 weeks old at initiation of dosing)
Number of animals: 60/sex/dose
Duration: 104 weeks
Route: Oral (gavage)

Dose Levels: 0 (control; vehicle - 5 ml/kg/day), low (5 ml/kg/day), mid (5 ml/kg/day) and high (5 ml/kg/day). The vehicle will be 1% carboxymethylcellulose aqueous solution. The low, mid and high dose levels will be determined from the results of the 90 day dose range study conducted in CD-1 mice.

Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 2X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14; once every 4 weeks thereafter; at week 104; and at termination.
5) *Food consumption* - 1X/week for weeks 1-13; then at ~3 month intervals unless health status or body weight changes dictate otherwise.
6) *Clinical pathology* - performed at week 52 and at terminal kill (week 104). There will be 10 mice/sex/group tested and collection will be from the same animals at each interval, if possible. The measurements will consist of differential leukocyte and cell morphology, erythrocyte count and leukocyte count.
7) *Gross necropsy* - performed at study termination
8) *Organ weights* - performed at study termination
9) *Histopathological examination* - A full range of organs and tissues will be preserved from all dose groups. All of the tissues will be examined from the control and high dose groups. Gross lesions will be examined microscopically from all animals. In addition, the lungs, liver and kidneys will be examined microscopically from all low and mid dose animals. Target organs noted at the high dose will be examined microscopically from all animals.

Comments: It is recommended that the sponsor not conduct clinical pathology assessment at week 104 because the results will be confounded by age related toxicities. It is recommended that the blood be drawn from a satellite group of animals (not the animals to be used for the main study) for the week 54 clinical pathology assessment. It is recommended that the sponsor conduct histopathological analysis on all of the tissues obtained from all of the dose groups in the carcinogenesis study. The sponsor should be advised that the mouse carcinogenicity protocol should be resubmitted after the dose levels are determined from the results of the 90 day dose range study in mice. In addition, the sponsor should be informed that the results of the 90 day dose range study in mice and the protocol for the 2 year carcinogenicity study in mice will be submitted to the executive CAC committee for their recommendations on the protocol. The results of their recommendation will be shared with the sponsor. In general, the proposed mouse carcinogenicity protocol appears to be adequate provided that the sponsor include the recommendations discussed above.

2) 104 week rat oncogenicity

Title: Oncogenicity Study of Thalidomide in Rats
Protocol number: 7089E
Performing organization: [ ]
Species/Strain: Fisher (F-344) rats (6-8 weeks old at initiation of dosing)
Number of animals: 70/sex/dose
Duration: 104 weeks
Route: Oral (gavage)
Dose Levels: 0 (control; vehicle - 5 ml/kg/day), low (5 ml/kg/day), mid (5 ml/kg/day) and high (5 ml/kg/day). The vehicle will be 1% carboxymethylcellulose aqueous solution. The low, mid and high dose levels will be determined from the results of the 90 day dose range study conducted in Fisher rats.
Dosing Schedule: The test article will be administered once a day and seven days a week.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 2X/day (cageside observations for toxicity)
3) *Physical examinations* - 1X/week (information will be recorded on the time of onset, location, size, appearance and progression of each grossly visible or palpable mass)
4) *Body weights* - Prior to treatment; 1X/week for weeks 1-14; once every 4 weeks thereafter; at week 104; and at termination.
5) *Food consumption* - 1X/week for weeks 1-13; once every four weeks thereafter; and at week 104.
6) *Clinical pathology* - performed at weeks 52, 78 and at terminal kill (week 104). There will be 10 rats/sex/group tested and collection will be from the same animals at each interval, if possible. The measurements will consist of differential leukocyte and cell morphology, erythrocyte count and leukocyte count.
7) *Gross necropsy* - performed at study termination.
8) *Histopathological examination* - A full range of organs and tissues will be preserved from all dose groups. All of the tissues will be examined for the control and high dose groups. Gross lesions will be examined microscopically from all animals. In addition, the lungs, liver and kidneys will be examined microscopically from all low and mid dose animals. Target organs noted at the high dose will be examined microscopically from all animals.

Comments: It is recommended that the sponsor not conduct clinical pathology assessment at week 104 because the results will be confounded by age related toxicities. It is recommended that the sponsor conduct histopathological analysis on all of the tissues obtained from all of the dose groups in the carcinogenesis study. The sponsor should be advised that the rat carcinogenicity protocol should be resubmitted after the dose levels are determined from the results of the 90 day dose range study in rats. In addition, the sponsor should be informed that the results of the 90 day dose range

study in rats and the protocol for the 2 year carcinogenicity study in rats will be submitted to the executive CAC committee for their recommendations on the protocol. The results of their recommendation will be shared with the sponsor. In general, the proposed mouse carcinogenicity protocol appears to be adequate provided that the sponsor include the recommendations discussed above.

3) Reproductive toxicity dose range finding study in male rabbits

Title: Oral (stomach tube) Dosage-Range Study of Thalidomide in Male Rabbits
Protocol number: 2103-001Pa
Performing organization: [ ]
Species/Strain: New Zealand White Rabbits (5-7 months; 2.5 - 5.5)
Number of animals: 5/sex/dose (only male rabbits will receive the test article)
Route: Oral (gavage)
Dose Levels: 0 (control; vehicle - 10 ml/kg/day); 30 mg/kg/day (10 ml/kg of 3 mg/ml stock); 150 mg/kg/day (10 ml/kg of 15 mg/ml stock); 300 mg/kg/day (10 ml/kg of 30 mg/ml stock); and 500 mg/kg/day (10 ml/kg of 50 mg/ml stock). The vehicle will be 1% carboxymethylcellulose aqueous solution. The dose levels were selected by the sponsor on the basis of published results of developmental toxicity studies of thalidomide in the rabbit.
Dosing Schedule: Male rabbits will be given the test article once daily beginning 14 days before mating and continue until the day before sacrifice (after a total of 42 days of dosing). Female rabbits will not be given the test article.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 2X/day for male rabbits; Days 0, 6, 13, 18 and 20 of presumed gestation for female rabbits.
3) *Body weights* - 1X/day for male rabbits; Days 0, 6, 13, 18 and 20 of presumed gestation for female rabbits.
5) *Food consumption* - 1X/day
6) *Toxicokinetics* - Toxicokinetic blood samples will be collected on the first and last day of dosage from all male rabbits. On the first day of dosage, samples will be collected at ~1, 2, 4, 8 and 16 hours postdosage. On the last day of dosage, samples will be collected immediately prior to dosage, and at ~1, 4, 8 and 16 hours postdosage.
7) *Caesarean-sectioning observations* - Female rabbits will be Caesarean-sectioned on day 20 of presumed gestation. The rabbits will be examined for the number and distribution of: corpora lutea, implantation sites, viable and nonviable embryos.
8) *Sperm Evaluation* - Samples of semen will be collected from each male rabbit and analyzed for motility and count following test article administration on the last day of dosage (day 28 of dosage).

9) *General necropsy information* - All rabbits will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.

10) *Male rabbit necropsy* - Male rabbits will be sacrificed on the day following the completion of the 28 day dosage period. A gross necropsy of the thoracic, abdominal and pelvic viscera will on performed on all male rabbits. The testes and epididymides will be removed, individual organ weights will be recorded and the organs will be preserved for histological examination. Testes and epididymides of control and high dosage group rabbits will be examined histologically.

13) *Female rabbit necropsy* - Female rabbits will be Caesarean-sectioned on day 20 of presumed gestation and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. Uteri of apparently nonpregnant does will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Comments: It is recommended that the sponsor include a full hematological and clinical chemistry profile measurements for male rabbits at appropriate time points in this study (i.e. day 7 and study termination). It is recommended that the sponsor evaluate mating performance in this study. Otherwise, the protocol outlined for the dose range study appears to be adequate.

4) Reproductive toxicity dose range finding study in female rabbits

Title: Oral (stomach tube) Dosage-Range Study of Thalidomide in Female Rabbits
Protocol number: 2103-001Pb
Performing organization: [redacted]
Species/Strain: Female New Zealand White Rabbits (5-7 months; 2.5 - 5.5 kg)
Number of animals: 5/dose
Route: Oral (gavage)
Dose Levels: 0 (control; vehicle - 10 ml/kg/day); 30 mg/kg/day (10 ml/kg of 3 mg/ml stock); 150 mg/kg/day (10 ml/kg of 15 mg/ml stock); 300 mg/kg/day (10 ml/kg of 30 mg/ml stock); and 500 mg/kg/day (10 ml/kg of 50 mg/ml stock). The vehicle will be 1% carboxymethylcellulose aqueous solution. The dose levels were selected by the sponsor on the basis of published results of developmental toxicity studies of thalidomide in the rabbit.
Dosing Schedule: Female rabbits will be given the test article once daily beginning 14 days before artificial insemination (female rabbits will be artificially inseminated using spermatozoa from a single proven male breeder) and continue until the day before sacrifice on day 34 of presumed gestation (female rabbits that do not deliver a litter) or on day 7 postpartum (female rabbits that deliver a litter). Note: F1 generation pups will not be directly given the test article but may be possibly exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day
3) *Body weights* - 1X/day
5) *Food consumption* - 1X/day
6) *Toxicokinetics* - Toxicokinetic blood samples will be collected on the first day of dosing and on days 8 and 19 of gestation. On the first day of dosage, samples will be collected at ~1, 2, 4, 8 and 16 hours postdosage. On days 8 and 19 of gestation, samples will be collected immediately prior to dosage, and at ~1, 4, 8 and 16 hours postdosage.
7) *Duration of gestation* - The duration of gestation will be calculated from day 0 of presumed gestation to the day of delivery for the first pup.
8) *Fertility parameters* - The following measurements will be obtained for rabbits assigned to natural delivery: Fertility Index (percentage of matings that result in pregnancies), Gestation Index (percentage of pregnancies that result in birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of doe and litter during the postpartum period, viability indices (percentage of kits born that survive 4 and 7 days).
9) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.
10) *Female rabbit necropsy* - Female rabbits will be sacrificed after completion of the seven day postpartum period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. The number and distribution of implantation sites will be recorded for all female rabbits. Dams with no surviving pups will be sacrificed after the last pup is found dead or missing. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for each female rabbit.
14) *F1 generation measurements* - Litters will be observed for dead pups at least twice daily. Clinical observations will be conducted on postpartum days 4 and 7. Body weights will be measured on postpartum days 4 and 7. Pups will be sacrificed on postpartum day 7 and examined for gross lesions. Necropsy will include a single cross-section of the head at the level of the frontal parietal suture and examination of the cross sectioned brain for apparent hydrocephaly. Pups that die prior to postpartum day 7 will be examined to try to determine the cause of death.

Comments: It is recommended that the sponsor include a full hematological and clinical chemistry profile measurements for female rabbits at appropriate time points in this study (i.e. day 7 and study termination). Otherwise, the protocol outlined for the dose range study appears to be adequate.

5) Segment I reproductive toxicity study in rabbit

Title: Oral (gavage) fertility and general reproduction toxicity study of thalidomide in rabbits
Protocol number: 2103-002
Purpose: The purpose of this study is to test for toxic effects/disturbances resulting from thalidomide treatment of male and female New Zealand White rabbits before mating and through gestation. This study evaluates ICH Harmonized Tripartite Guideline stages A through D of the reproductive process and should detect effects on tubal transport, implantation, and development of the embryos and fetuses of female rabbits and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rabbit reproductive organs.
Performing organization: [ ]
Species/Strain: New Zealand White rabbit (5-7 months; 2.5 - 5.5 kg)
Number of animals: 20/sex/dose
Route: Oral (gavage)
Dose Levels: There will be a control (vehicle), low, mid and high dose group. The dose levels will be selected on the basis of the results of the reproductive toxicity dose range finding studies in rabbit. The vehicle will be 1% carboxymethylcellulose aqueous solution.
Dosing Schedule: Male rabbits will be given the test article once daily beginning 14 days before mating and continue through the day before sacrifice (a total of 56 days of dose administration). Female rabbits will be given the test article once daily beginning 14 days before mating and continue until the day before sacrifice on day 29 of presumed gestation.
Assessments:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 2X/day
3) *Body weights* - 1X/day
4) *Food consumption* - 1X/day
5) *Caesarean-sectioning observations* - Rabbits will be Caesarean-sectioned on day 29 of presumed gestation. Placentae that appear abnormal (size, color or shape) will be noted in the raw data. The rats will be examined for number and distribution of: corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions.
6) *Fetal observations* - Body weight, sex and gross external alterations will be determined for each fetus. Fetuses with gross external alterations will be preserved in neutral buffered 10% formalin. All other fetuses will be discarded after examination.
7) *Sperm evaluation* - Samples of semen will be collected from each male rabbit and analyzed for motility and count following test article administration on the last day of dosage.

8) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.

9) *Male rabbit necropsy* - Male rabbits will be sacrificed following the completion of the 56 day dosage period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed on all male rats. The testes and epididymides will be removed, individual organ weights will be recorded and the organs will be preserved for histological examination. Testes and epididymides of control and high dosage group rabbits will be examined histologically.

10) *Female rabbit necropsy* - Female rabbits will be sacrificed on day 29 of presumed gestation. A Caesarean-section will be performed at that time and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rats. The number and distribution of implantation sites will be recorded for each female rabbit. Uteri of apparently nonpregnant rats will be stained with 10% ammonium sulfide to confirm the absence of implantation sites. All ovaries will be preserved for possible future evaluation.

Comments: It is recommended that the sponsor evaluate mating performance in this study. Otherwise, the protocol outlined for the Segment I study appears to be adequate. The sponsor is advised to submit the anticipated doses to be used for this study along with the results of the reproductive toxicity dose range finding studies in support of this dose selection.

6) Segment III reproductive toxicity study in rabbit

Title: Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of thalidomide in rabbits, including a postnatal reproductive evaluation

Protocol number: 2103-001

Purpose: The purpose of this study is to detect adverse effects of thalidomide treatment of female New Zealand White rabbits from the closure of the hard palate through lactation and weaning on gestation, parturition, lactation and maternal behavior in female rabbits and on the development of the offspring of the treated female rabbits. This study evaluates ICH Harmonized Tripartite Guideline stages D through F of the reproductive process and does not include an evaluation of Caesarean-delivered fetuses (stages C and D), because this evaluation is performed in a supplementary study. Because manifestations of effects induced during this period may be delayed, observations will be continued through sexual maturity of the F1 generation rabbits.

Performing organization: [ ]

Species/Strain: Female New Zealand White rabbit (5-7 months; 2.5 - 5.5 kg)

Number of animals: 20 mated females/dose

Route: Oral (gavage)

Dose Levels: There will be a control (vehicle), low, mid and high dose group. The dose levels will be selected on the basis of the results of the reproductive toxicity dose range

finding studies in rabbit. The vehicle will be 1% carboxymethylcellulose aqueous solution.

Dosing Schedule: Female rabbits will be given the test article once daily from day 18 of presumed gestation through day 28 postpartum or day 33 of presumed gestation (rabbits that do not deliver a litter). F1 generation pups will not be directly given the test article, but may be possibly exposed to the test article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Assessments for Fo Generation:

1) *Mortality/Morbidity* - 2X/day
2) *Clinical observations* - 1X/day
3) *Body weights* - 1X/day
4) *Food consumption* - 1X/day
5) *Milk sample collection* - Milk samples will be collected from female rabbits once ~4 hours postdosage between days 8 and 14 postpartum.
6) *Natural delivery* - Female rabbits will be evaluated for the following criteria during natural delivery: 1) clinical observations (including the time each pup is delivered) during parturition (between 0700 and 1900 hours EST); 2) duration of gestation (day 0 of presumed gestation to the time the first pup is delivered; 3) length of parturition (time of delivery of last pup minus the time of delivery of the first pup divided by N-1 pups in each litter); 4) litter size (defined as all pups delivered); 5) viability indices (percentage of pups born that survive 4 and 7 days); and 6) lactation index (percentage of pups born that survive 28 days).
8) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. All other tissues will be discarded unless specifically cited below.
9) *Female rat necropsy* - Female rabbits will be sacrificed after the 28 day postpartum period. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for all female rabbits. The number and distribution of implantation sites will be recorded for each female rabbit. Female rabbits that do not deliver a litter will be sacrificed on day 34 of presumed gestation and examined for gross lesions. Uteri will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Assessments for F1 Generation:

1) *Viability* - Preweaning period: Litters will be observed for dead pups at least twice daily. The pups in each litter will be counted 1X/day. Postweaning period: 2X/day.
2) *Clinical observations* - Preweaning period: 1X/day. Postweaning period: 1X/week.

3) *Body weights* - Preweaning period: Days 4, 7, 14, 21 and 28 postpartum. Postweaning period: 1X/week. Presumed gestation period: Days 0, 7, 10, 14, 17, 21, 14 and 29 (female rabbits only)
4) *Food consumption* - Preweaning period: not recorded. Postweaning period: 1X/week
5) *Reproductive evaluation* - Twelve male and twelve female rabbits per dose group will be selected for reproductive evaluation at five months of age. One male rabbit and one female rabbit will be paired for mating and each pair will be monitored continuously until mating is confirmed by observation. Following mating, the female rabbit will be returned to it's individual cage. The day of mating will be designated day 0 of presumed gestation. Female rabbits will be Caesarean sectioned on day 29 of presumed gestation. The rabbits will be examined for number and distribution of: corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus will be weighed and examined for sex and gross external alterations. Representative photographs of fetal alterations will be taken. Fetuses will be tagged with identification noting study number, litter number, uterine distribution and fixative, and retained for possible future evaluation.
6) *General necropsy information* - All rabbits and live fetuses will be sacrificed upon study termination. Gross lesions will be preserved for possible future evaluation. Selected F1 rabbits for reproductive evaluation will have sections of the sciatic, tibial, fibular and sural nerves excised and preserved for possible future histological evaluation. All other tissues will be discarded unless specifically cited below.
7) *Pup necropsy* - Pups found dead or sacrificed because of moribundity will be examined for gross lesions and for the cause of death or the moribund condition. Gross lesions will be preserved for possible future evaluation. All pups culled on day 28 postpartum (pups not selected for continued observation) will be sacrificed and examined for gross lesions. The gross lesions will be preserved for possible future evaluation. Necropsy will include a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.
8) *Male rabbit necropsy* - Male rabbits will be sacrificed after completion of mating. A gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. Testes and epididymides of male rabbits will be excised and paired organ weights will be recorded and the organs will be preserved for possible future evaluation.
9) *Female rabbit necropsy* - Female rabbits will be sacrificed on day 29 of presumed gestation and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed for each rat. Uteri of apparently nonpregnant rabbits will be stained with 10% ammonium sulfide to confirm the absence of implantation sites.

Comments: It is recommended that the sponsor evaluate a measure of sexual maturation in this study. It is also recommended that the sponsor evaluate some parameters of development in this study (i.e. measurements of learning capacity, physical strength, and motor coordination). Otherwise, the protocol outlined for the Segment III study appears to be adequate. The sponsor is advised to submit the anticipated doses to be used for this study along with the results of the reproductive toxicity dose range finding studies in support of this dose selection.

Discussion:

The sponsor has conducted the following studies for this NDA submission: 1) pharmacokinetic studies in mouse, rat and dog, 2) 14 day repeat dose toxicity studies in mice and rats, 3) 90 day repeat dose toxicity study in rats, 4) 28 day repeat dose toxicity study in dogs, 5) 52 week repeat dose toxicity study in dogs (6 month interim report submitted), and 6) genetic toxicity tests (the Ames test, a clastogenicity assay and an *in vivo* micronucleus test). The sponsor will need to submit the results for the 90 day repeat dose toxicity study in mice and the final report for the 52 week repeat dose toxicity study in dogs after completion of these studies. The sponsor has also submitted the following phase IV protocols: 1) 104 week mouse oncogenicity study, 2) 104 week rat oncogenicity study, 3) Reproductive toxicity dose range finding study in male rabbits, 4) Reproductive toxicity dose range finding study in female rabbits, 5) Segment I reproductive toxicity study in rabbit, and 6) Segment III reproductive toxicity study in rabbit.

Pharamacokinetic profiles for thalidomide after single or repeat dose administration in rats, mice and dogs demonstrate an increase in C_{max} and AUC with increased dose which is non-linear. This suggests that the absorption of thalidomide delivered via the oral route is saturable and may be related to the low intrinsic solubility of thalidomide in aqueous solutions. There is no evidence from any of the nonclinical pharmacokinetic studies that there is accumulation of thalidomide after repeat dose administration even though the elimination rate does increase with increased dose. A potential explanation for this may be that the rate of spontaneous hydrolysis of thalidomide is so rapid that accumulation after repeat dose administration of thalidomide is not possible. However, it may be possible that an active metabolite (or spontaneous hydrolysis product) could have accumulated after repeat dose administration, but since the sponsor only measured parent thalidomide in the pharmacokinetic studies this can not be determined for the nonclinical studies submitted for the NDA. It would have been helpful if the sponsor conducted a nonclinical radioactive distribution study to characterize the tissue distribution of thalidomide (and metabolites/spontaneous hydrolysis products) after single and repeat dose administration. In addition, a more comprehensive pharmacokinetic study including measurement of parent thalidomide, potential metabolites and spontaneous hydrolysis products, after single and repeat dose administration would have generated more complete data to determine if one of the potential metabolites and/or a spontaneous hydrolysis product may be responsible for any toxic effects associated with thalidomide administration.

The maximum achievable AUC obtained on a consistent basis in all species was ~400 µg*hr/ml. This maximum nonclinical AUC is ~11X the proposed maximum clinical dose of thalidomide (400 mg/day; AUC ~36.4 ± 9.55 µg*hr/ml) and ~21X the commonly prescribed clinical dose of thalidomide in ENL (200 mg/day; ~18.9 ± 3.28 µg*hr/ml). It is important to note that there was considerable variation in the pharmcokinetic parameters calculated from study to study within the same species. Therefore, it is recommended that the sponsor conduct adequate pharmacokinetic sampling throughout the proposed phase IV nonclinical studies to assure that adequate amounts of thalidomide are absorbed systemically to accurately assess the toxicity profile of thalidomide in these nonclinical studies.

Results from repeat dose toxicity nonclinical studies conducted in this NDA suggest that rodents are more sensitive to thalidomide toxicity than dogs. Toxicities observed in mice after 14

day repeat dose administration include: 1) minimal centrilobular hepatocellular nuclear pleomorphism with a corresponding increase in liver weight (dose dependent in male mice, but not in female mice), 2) treatment related effect on the eyes (mainly cataracts; not dose-dependent), and 3) formation of discolored urine. Toxicities observed in mice after 90 day repeat dose administration include: 1)centrilobular hepatocellular hypertrophy with a corresponding increase in liver weight (dose dependent increase in severity in male mice, only observed in high dose female mice), 2) a dose dependent decreasing trend in lymphocyte counts, and 3) formation of discolored urine. The potential treatment related effects on the eyes (cataract formation) observed in the 14 day repeat dose study in mice were not verified in this 90 day repeat dose toxicity study. The formation of corneal crystals was seen in three control animals and one animal from each of the dose groups. The significance of this finding is unclear since there was a rather high incidence of corneal crystal formation observed in control animals. It is requested of the sponsor to submit additional information concerning the historical control data for CD-1 mice and the formation of corneal crystals. In addition, it would be helpful for the sponsor to provide a potential explanation for: 1) the formation of cataracts in the 14 day repeat dose toxicity study and not in the 90 day repeat dose toxicity study performed in mice and 2) the formation of corneal crystals in the 90 day repeat dose toxicity study and not in the 14 day repeat dose toxicity study performed in mice.

There were two neoplasms observed in the 90 day repeat dose toxicity study performed in CD-1 mice. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. I do not concur with the contract lab's assessment that both tumors were incidental and not related to the administration of the test article since these were isolated lesions and are relatively common spontaneous neoplasms in CD-1 mice. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are relatively common spontaneous neoplasms in CD-1 mice, but I would anticipate that these tumors are relatively uncommon in CD-1 mice at this early of a time in their life span. It is requested of the sponsor to submit additional information on the historical control data for CD-1 mice to validate the claim that the two types of tumors observed in this study are relatively common spontaneous neoplasms in CD-1 mice. In particular, special attention should be paid to clarifying at what timepoint in the life span of the CD-1 mice are alveolar-bronchiolar adenomas and uterine stromal polyps observed and what is their frequency level. One potential possibility for the presence of the alveolar-bronchiolar adenoma could be due to a murine virus infection of that particular animal. It is requested of the sponsor to provide additional information on the health status of the CD-1 mice used in this 13 week repeat dose toxicity study. Particular attention should be paid to the results of the 2 year carcinogenicity assay performed in mice that the sponsor will conduct as a phase IV commitment. The results from the 2 year carcinogenicity assay may help to determine the biological relevance of the two types of tumors observed in this 13 week repeat dose toxicity study. The observed liver toxicity in this experiment was of potential concern because a NOTEL could not be established for the male mice. However, this may be a species specific effect because this type of hyperproliferative effect has been seen in mouse liver after other xenobiotic treatment regimens.

Toxicities observed in the rats after 14 day repeat or 90 day repeat dose administration include: 1) dose dependent decrease in body weight (more pronounced in male rats), 2) dose

dependent decrease in platelets and mild leukopenia, 3) dose dependent effect on thyroid function (decreased levels of total and free T4 levels), and 4) treatment related decrease in thymus weight. The results of the 90 day repeat dose toxicity study in rats indicated that there was not a NOTEL for the effects on body weight loss. This may be of potential clinical concern but once again may also be a species specific effect since this drastic decrease in body weight was not observed in either mice or dogs.

Relatively few toxicities were noted in either the 28 day repeat dose toxicity study in dogs or the 6 month interim report for the 52 week repeat dose toxicity study in dogs. The major observation noted in both studies was the appearance of green urine and white material in the feces after thalidomide treatment. The frequency of both incidences increased with increased dose. The white material in the feces was due to unabsorbed thalidomide. The presence of discolored urine in the mouse and dog study may indicate the metabolic formation of a chromaphore from thalidomide that is excreted in the urine. Red and blue substances have been reported from simple hydrolysis of thalidomide (Schumacher, H. Smith, R.L., and Williams, R.T. 1965a. The metabolism of thalidomide: The spontaneous hydrolysis of thalidomide in solution Brit. J. Pharmacol. 25: 324-337) as well as excreted in the urine of laboratory animals treated with thalidomide (Schumacher, H., Smith, R.L., and Wiliams, R.T. 1965b. The metabolism of thalidomide: The fate of thalidomide and some of its hydrolysis products in various species. Brit. J. Pharmacol. 25: 338-351). Additional toxicities noted in the 6 month interim report included: 1) treatment related enlargement of mammary tissue (no histopathological confirmation of this result), 2) dose dependent increase in discoloration of the cranial bones (no histopathological confirmation of this result), and 3) a slight axonal swelling with loss of neruofilaments observed in high dose male dogs which may be related to thalidomide treatment. The results from the final report for the 52 week repeat dose toxicity study in dogs will be of paramount importance to determine if any of these observations are significant. It is important to note that the maximum AUC obtained in this study (~ 100 µg*hr/ml) is substantially lower (~¼X) than was obtained in the 7 day repeat dose pharmacokinetic study (~400 µg*hr/ml) in dogs described above. The AUC value for the 43 mg/kg/day group (~ 10 µg*hr/ml) was ~½X that observed clinically after a 200 mg/day dose of thalidomide (~19 µg*hr/ml). This raises some concern over whether the doses used in this 1 year study will be high enough to accurately characterize the chronic toxicity profile for thalidomide in dogs. It will be important to see the pharmacokinetic results from the 6 month and 1 year time points for a more accurate assessment of the adequacy of the dose range used in this 1 year dog study.

The results from the genetic toxicology studies demonstrate that thalidomide is not mutagenic in the Ames test or clastogenic in both *in vitro* and *in vivo* tests. There is some question concerning the stability of thalidomide in the two *in vitro* mutagenicity tests due to the rapid spontaneous hydrolysis of thalidomide at neutral pH. It would provide additional assurance about the lack of genetic toxicology for thalidomide if an *in vivo* mutagenicity study were conducted by the sponsor.

In general, the submitted nonclinical protocols for phase IV carcinogenicity studies in rats and mice and reproductive toxicity studies (Segment I study in rabbits to determine effects of thalidomide on fertility and Segment III study in rabbits to determine effects of thalidomide when

administered in late stage pregnancy) appear to be adequate. There were a few minor recommendations for these protocols that are outlined in the recommendations section below.

The nonclinical portion of this NDA submission is complete. Based on the results of the nonclinical studies submitted with this NDA and the proposed phase IV nonclinical protocols, I recommend approval of thalidomide for the treatment of erythema nodosum leprosum from a pharmacological/toxicological perspective.

LABEL REVIEW:

Note: The contents of this label review section are a direct copy of the information that the sponsor submitted in electronic format on 2-3-97 (which is the most recent label submitted to this NDA). In this labeling review I have included my recommendations to the medical officer in parentheses as italics and shadowing portions. My actual recommendations for label changes are noted as either strikeout of the text for deletions or shadowing of text for additions. It is important to note that the labeling section that was submitted with the NDA contains considerably inadequate clinical information and very little pharm/tox information. I anticipate that there will be numerous rounds of revision for the submitted label for this NDA particularly in light of the fact that the sponsor has not decided whether thalidomide will be used as a monotherapy or adjunctive therapy for the treatment of ENL.

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____ § 552(b)(4) Trade Secret / Confidential

____ § 552(b)(5) Deliberative Process

✓ § 552(b)(4) Draft Labeling

REGULATORY CONCLUSION:

The submitted nonclinical studies are adequate to determine the nonclinical toxicity profile for thalidomide. The submitted nonclinical protocols are adequate (with a few minor recommendations listed below) to assess the carcinogenic and reproductive (male fertility and late stage pregnancy) toxicity risk for thalidomide from a pharmacological/toxicological perspective. The conduct of the submitted nonclinical protocols should support the phase IV commitments that were agreed upon with the sponsor from a pharmacological/toxicological perspective. Based on the results of the nonclinical studies submitted with this NDA and the proposed phase IV nonclinical protocols, I recommend approval of thalidomide for the treatment of erythema nodosum leprosum from a pharmacological/toxicological perspective.

RECOMMENDATIONS:

The following information should be relayed to the sponsor:

1) It is requested that the sponsor submit additional information concerning the historical control data for CD-1 mice and the formation of corneal crystals. In addition, it would be helpful for the sponsor to provide a potential explanation for: a) the formation of cataracts in the 14 day repeat dose toxicity study and not in the 90 day repeat dose toxicity study performed in mice and b) the formation of corneal crystals in the 90 day repeat dose toxicity study and not in the 14 day repeat dose toxicity study performed in mice.

2) There were two neoplasms observed in the 13 week repeat dose toxicity study performed in CD-1 mice. A low dose male had a small alveolar-bronchiolar adenoma involving the lung and a high dose female had a uterine stromal polyp. Tumor findings are quite uncommon in a 13 week repeat dose toxicity study. It may be true that the two types of observed tumors are relatively common spontaneous neoplasms in CD-1 mice, but I would anticipate that these tumors are relatively uncommon in CD-1 mice at this early of a time in their life span. It is requested that the sponsor submit additional information on the historical control data for CD-1 mice to validate the claim that the two types of tumors observed in this study are relatively common spontaneous neoplasms in CD-1 mice. In particular, special attention should be paid to clarifying at what time point in the life span of the CD-1 mice are alveolar-bronchiolar adenomas and uterine stromal polyps observed and what is their frequency level. One potential possibility for the presence of the alveolar-bronchiolar adenoma could be due to a murine virus infection of that particular animal. It is requested of the sponsor to provide additional information on the health status of the CD-1 mice used in this 13 week repeat dose toxicity study.

3) There is some concern over the AUC values obtained in the 52 week repeat dose toxicity study in dogs. The maximum AUC obtained in this study (~ 100 μg*hr/ml) is substantially lower (~ ¼X) than was obtained in the 7 day repeat dose pharmacokinetic

study (~400 μg*hr/ml) performed in dogs. It is requested that the sponsor submit any additional information that they may have to provide an explanation for this observation. In particular, information concerning the status of the dogs fed or fasted state prior to dose administration would be quite useful. In dogs, the pH in the stomach varies according to how recently the dogs have eaten and this could have a dramatic effect on the rate of spontaneous hydrolysis of thalidomide.

4) There is some concern over the stability of thalidomide (due to spontaneous hydrolysis) under the assay conditions for the *in vitro* genetic toxicology studies that investigated the potential for thalidomide to induce mutations. It is unclear as to the stability of thalidomide in the media used to conduct the two *in vitro* genetic toxicology studies. It is requested that the sponsor submit information to the agency about the stability of thalidomide under the conditions of the two *in vitro* genetic toxicology studies conducted for thalidomide.

The following recommendations should be relayed to the sponsor:

1) It is recommended that the sponsor make the following modifications to the two carcinogenicity (rat and mouse) protocols: a) delete the clinical pathology assessment at week 104 due to these results may be confounded by age related toxicities, b) draw blood from a satellite group of mice (not the animals to be used for the main study) for the week 54 clinical pathology assessment, and c) conduct histopathological examination of all of the tissues from all of the dose groups in both carcinogenicity assays.

2) It is recommended that the sponsor resubmit the two carcinogenicity (rat and mouse) protocols with the results of their respective 90 day dose range studies to support the dose selection for these studies. The sponsor should be advised that the two carcinogenicity protocols, along with their respective 90 day dose range studies, will be submitted to the executive Carcinogenicity Assessment Committee (CAC) for evaluation and recommendations. The recommendations from the executive CAC evaluation of the two carcinogenicity protocols will be shared with the sponsor.

3) It is recommended that the sponsor include full hematological and clinical chemistry profile measurements in both of the reproductive toxicity dose range finding studies in male and female rabbits at appropriate time points in this study (i.e. day 7 and study termination). It is recommended that the sponsor evaluate mating performance in the reproductive toxicity dose range finding study in male rabbits.

4) It is recommended that the sponsor evaluate mating performance in the Segment I reproductive toxicity study in rabbit.

5) It is recommended that the sponsor evaluate a measure of sexual maturation in the Segment III reproductive toxicity study in rabbit. It is also recommended that the sponsor evaluate some parameters of development in this study (i.e., measurements of learning capacity, physical strength, and motor coordination).

6) It is recommended that the sponsor resubmit the Segment I and III reproductive toxicity protocols after completion of the reproductive toxicity dose range finding studies to support the dose selection for these studies.

Barbara Ann Hill

Barbara Ann Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 20-765 (original submission)
HFD-340
HFD-540
HFD-540/TOX/AJACOBS
HFD-540/PHARM/HILL
HFD-540/MO/VAUGHN
HFD-540/CHEM/DECAMP
HFD-725/BIOSTAT/GAO
HFD-880/BIOPHARM/BASHAW
HFD-540/PM/WHITE
C:WPFILES/NDAS/NDA20725/20725000.WPD

Concurrence Only:
HFD-540/DD/JWILKIN
HFD-540/TOX/AJACOBS 6.9. 6/12/14