

Dose selection criteria:

Basis of dose selection: Selection of doses for the micronucleus assay was based on the toxicity of the test article but did not exceed 2000 mg/ kg.

Range finding studies: The range finding study (also toxicity study) was performed using up to five test article doses, (0.5, 1, 5, 7.5 and 10 mg/kg) each containing up to five male and five female mice. Dose administration was via one single IV injection. The high dose for the micronucleus test may be 50% to 80% of the LD<sub>50/3</sub> (the dose required to kill 50% of the animals within 3 days after administration) or the maximum tolerated dose but did not exceed 2000 mg/ kg. Two additional doses were tested, one- half and one- fourth of the high dose.

Test agent stability: Stable up to 12 months.

Controls:

Negative controls: Vehicle control

Positive controls: Cyclophosphamide monohydrate at a dose of 30- 60 mg/ kg. CP will be administered by the same route as the test article.

Exposure conditions:

Incubation and sampling times: Bone marrow cells, collected 24 and 48 hours after IV injection of PS-341, were examined microscopically for micronucleated polychromatic erythrocytes. However, in the positive control group bone marrow cells were collected 24 hours after administration only.

Doses used in definitive study:

Range-finding study (also toxicity study): 0.5, 1, 5, 7.5, and 10 mg/kg (injection volume: 10 ml/kg).

Definitive micronucleus study: 0.25, 0.5, 1 mg/kg (injection volume: 10 ml/kg).

Study design: The assay was performed in two phases. The first phase, designed to assess toxicity of the test article and set dose levels for the definitive study, consisted of a toxicity study. The second phase, the definitive micronucleus study, was designed to evaluate the potential of PS- 341 to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female mice.

Analysis:

No. of replicates: Not applicable.

Animal observations:

Mice were observed after dose administration (single IV injection) and each working day thereafter for 3 days for clinical signs of chemical effect. Body weights were recorded prior to dose administration and at 1 and 3 days after dose administration.

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Criteria for positive results: For a test article to be evaluated positive, it must cause a dose responsive increase in micronucleated polychromatic erythrocytes and one or more doses were statistically elevated relative to the vehicle control ( $p \leq 0.05$ , Kastenbaum-Bowman Tables) at any sampling time.

**Summary of individual study findings:**

Study validity: The study has followed the criteria required for a valid test, because:

- Bone marrow cell exposure to PS- 341 was verified by the observation of the reduction of polychromatic erythrocytes relative to total erythrocytes. Reductions were observed in some of PS- 341- treated groups (e.g., in female mice reduced 5% at 0.25 mg/kg, and reduced 4% at 0.5 mg/kg), especially at the high dose (1 mg/kg) of PS- 341 (9- 46%).
- Mean incidence of micronucleated polychromatic erythrocytes (PCEs) did not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control: it was 0-0.3 per 1000 PCEs, and it was within the historical control data of the laboratory.
- Positive control, cyclophosphamide (50 mg/kg), induced a significant increase in micronucleated PCEs in both male and female mice ( $p \leq 0.05$ , Kastenbaum- Bowman Tables). It was also within the range of historical control data of the laboratory.

Study outcome:

- Clinical observations in the toxicity study:
  - Mortality: occurred within two days of dose administration. At dose levels of 5, 7.5, 10 mg/kg, all mice died regardless of sex or dose difference. No death occurred in the 0.5 or 1 mg/kg group.
  - Clinical signs:

Clinical Signs Following Dose Administration of PS-341 in ICR Mice Toxicity Study

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Mortality/Total Number of Animals Dosed	
		Males	Females	Males	Females
PS-341 0.5 mg/kg	*	0/5	0/5	0/5	0/5
1 mg/kg	Lethargy Piloerection	3/5 3/5	3/5 0/5	0/5	0/5
5 mg/kg	Lethargy Piloerection Eyes half opened Crusty eyes Hunched position	5/5 5/5 5/5 5/5 5/5	5/5 5/5 5/5 5/5 5/5	5/5	5/5
7.5 mg/kg	Lethargy Piloerection Eyes half opened Crusty eyes Hunched position	5/5 5/5 5/5 5/5 5/5	5/5 5/5 5/5 5/5 5/5	5/5	5/5
10 mg/kg	Lethargy Piloerection Eyes half opened Crusty eyes Hunched position Irregular breathing	5/5 4/5 5/5 4/5 4/5 4/5	5/5 4/5 5/5 4/5 4/5 1/5	5/5	5/5

\*No clinical signs observed, all animals appeared normal after dose administration

- In the definitive micronucleus study, 0.25, 0.5 and 1 mg/kg PS-341 was administered to mice via one single IV injection. No mortality occurred during the course of study. Clinical signs, which were noted on the day of dose administration, included: lethargy and eyes half opened in male and female mice at 1 mg/ kg. No clinical signs were observed at doses  $\leq$  0.5 mg/ kg PS- 341. All other mice treated with test or control articles appeared normal during the study.
- The number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes in PS- 341- treated groups was not statistically increased in either male or female mice, regardless of dose level or bone marrow collection time ( $p > 0.05$ , Kastenbaum- Bowman Tables) relative to the respective vehicle controls.
- Conclusion: Under the study conditions of the micronucleus assay, PS-341, up to 1 mg/kg via a single IV injection, did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of mice.
- Summary of the study (cited from the sponsor):

Table 4

Summary of Bone Marrow Micronucleus Analysis Using PS-341 in ICR Mice

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean $\pm$ SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean $\pm$ SD)	Number per PCEs Scored <sup>1</sup>
Mannitol in sodium chloride for injection, USP							
10 mg/kg	M	24	5	0.446 $\pm$ 0.03	—	0.1 $\pm$ 0.22	1 / 10000
	F	24	5	0.505 $\pm$ 0.01	—	0.3 $\pm$ 0.45	3 / 10000
PS-341							
0.25 mg/kg	M	24	5	0.482 $\pm$ 0.06	8	0.2 $\pm$ 0.45	2 / 10000
	F	24	5	0.481 $\pm$ 0.03	-5	0.1 $\pm$ 0.22	1 / 10000
0.5 mg/kg	M	24	5	0.450 $\pm$ 0.09	1	0.1 $\pm$ 0.22	1 / 10000
	F	24	5	0.486 $\pm$ 0.04	-4	0.3 $\pm$ 0.45	3 / 10000
1.0 mg/kg	M	24	5	0.241 $\pm$ 0.10	-46	0.3 $\pm$ 0.45	3 / 10000
	F	24	5	0.411 $\pm$ 0.06	-19	0.3 $\pm$ 0.45	3 / 10000
CP							
50 mg/kg	M	24	5	0.464 $\pm$ 0.04	4	21.7 $\pm$ 5.63	217 / 10000
	F	24	5	0.462 $\pm$ 0.03	-9	21.3 $\pm$ 3.01	213 / 10000
Mannitol in sodium chloride for injection, USP.							
10 mg/kg	M	48	5	0.446 $\pm$ 0.05	—	0.0 $\pm$ 0.00	0 / 10000
	F	48	5	0.540 $\pm$ 0.07	—	0.2 $\pm$ 0.27	2 / 10000
PS-341							
1.0 mg/kg	M	48	5	0.383 $\pm$ 0.08	-14	0.1 $\pm$ 0.22	1 / 10000
	F	48	5	0.494 $\pm$ 0.03	-9	0.3 $\pm$ 0.27	3 / 10000

PCE = Polychromatic erythrocytes

<sup>1</sup>Statistically significant,  $p \leq 0.05$  (Kastenbaum-Bowman Tables)

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**Genetic toxicology summary:**

Three *in vitro* tests (a [redacted] assays) and an *in vivo* test ([redacted] were performed to assess the genotoxicity of PS-341. All the tests were performed under GLP regulation and the test results were valid. Based on the test result, PS-341 was not mutagenic in [redacted] tests and the [redacted] test under the conditions of the assay. Although PS-341 did not induce numerical chromosome aberration in CHO cells, PS-341 was positive for the induction of structural chromosome aberration, with and without metabolic activation.

**Genetic toxicology conclusions:** PS-341 was positive for the induction of structural chromosome aberrations, with and without metabolic activation.

**VI. CARCINOGENICITY:**

**Studies not submitted**

UNIDENTIFIED

**VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:**

Reviewer: Kimberly Benson, Ph.D.

Study title: An intravenous injection range-finding teratology study of PS-341 in the Sprague-Dawley rat.

**Key study findings:**

- Three highest doses tested were maternally lethal, little evaluable data obtained.
- Lowest dose, 0.05 mg/kg/day (0.3 mg/m<sup>2</sup>/day) with an AUC<sub>(0-24)</sub> of 99 hr·ng/mL by day 17 of gestation, no maternal toxicity.
- Toxicokinetic data show non-linear increases in AUC<sub>(0-24)</sub> and C<sub>max</sub>.
- Pharmacodynamic data show 80-90% inhibition for PS-341 doses 30 minutes after the first drug administration on GD 6 in the LD group.

Study no.:	Project number 98172
Volume #, and page #:	Module 4; 4.2.3.5.2.1
Conducting laboratory and location:	[ ]
Date of study initiation:	27 November 2001
GLP compliance:	Compliance included and signed.
QA reports:	yes ( ) no (X)
Drug, lot #, and % purity:	PS-341, lot# D2-1-1, — ; purity not given
Formulation/vehicle:	D-Mannitol in 0.9% NaCl

**Methods:**

Species/strain:	Rat/Sprague Dawley CD (CrI:CD®[SD-7784]IGS BR)
Doses employed:	Vehicle control = 0 LD = 0.05 mg/kg/day MD1 = 0.10 MD2 = 0.15 HD = 0.20
Route of administration:	IV bolus injection; variable volume
Study design:	Segment II Study Females – Dosed once daily gestational day (GD) 6-17  Males – Not dosed
Number/sex/group:	6 ♀/group plus 12 satellite females for toxicokinetic and pharmacodynamic evaluation.
Parameters and endpoints evaluated:	Females – clinical signs, body weight, food consumption, at GD 13 – combined weight of uterus, uterine contents, ovaries and oviducts. Corpora lutea <sup>1</sup> count from both ovaries. Number of implantation sites, viable embryos and resorptions in each uterine horn.

Rate of pregnancies also calculated. Gross necropsy.  
 Fetuses – weight, detailed external examination, sex.  
 Toxicokinetics: Blood taken from satellite rats (3/sex/group) at 30 minutes, 1, 6 and 24 hours after dosing on GD 6 and 17.  
 Pharmacodynamics: Blood taken from satellite rats (3/sex/group) for toxicokinetics used for 20S proteasome activity analysis.

**Results:**

**Mortality:** HD – 6/6 rats died or euthanized in morbid condition – GD 7/8  
 MD2 – 6/6 rats died or euthanized in morbid condition – GD 7/8  
 MD1 – 5/6 rats died or euthanized in morbid condition – GD 8-18  
 LD – 0/6 rats died

**Clinical signs:** HD and MD2 – prior to death, ↓ activity, hunched posture, ↓ muscle tone, convulsions, abnormal breathing, ptosis, pale skin, we fur and/or yellow stain at urogenital area, and cold to touch.  
 MD2 – same as HD plus brown mucoid material on cage tray  
 MD1 – same as HD plus lack of grooming, discharge from eyes, ↓ fecal output.

**Body weight:** LD - no treatment-related effects  
 MD1 – surviving dams weight loss between GD 6 until euthanized, up to a decrease of 29% in body weight seen compared to pre-dosing weight.  
 MD1 – body weight gains ↓ by at least 128% when compared to body weight gains of control dams

**Food consumption:** LD – no treatment-related effects  
 MD1 – surviving dams ↓ food consumption compared to controls.  
 GD 6-9 = 80% ↓ compared to control  
 GD 9-12 = 73% ↓ compared to control  
 GD 12-15 = 31% ↓ compared to control  
 GD 15-18 = 38% ↓ compared to control

**In-life observations:** Number animals mated and number of pregnant females equal across groups

**Terminal and necroscopic evaluations:**

**Dams:** LD dams – ovarian and uterine parameters were all comparable to control.  
 MD1 – in the one dam that survived – comparable corpora lutea and implantation sites to control. But total resorption was seen – 3 early, 11 middle and 2 late.  
 Gross pathology

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LD – no treatment-related effects  
 MD1 – Adrenal enlargement – 2/6  
 Pale discoloration of carcass – 3/6  
 Emaciation – 4/6  
 Pale discoloration of the liver – 3/6  
 Depressed areas in the stomach – 2/6

Offspring: LD - No external malformations in the only group with viable fetuses.

LD - fetal weights comparable to control.

Toxicokinetics:

Text Table 1. Mean Values for the Toxicokinetic Parameters for PS-341

	0.05 mg/kg/day	0.10 mg/kg/day	0.15 mg/kg/day	0.20 mg/kg/day
<b>Gestation Day 6</b>				
AUC <sub>(0-24)</sub> (hr·ng/mL)	20.5	44.8	80.8	191
C <sub>(max)</sub> (ng/mL)	2.6	5.9	11.5	20.7
<b>Gestation Day 17</b>				
AUC <sub>(0-24)</sub> (hr·ng/mL)	99	NA	NA	NA
C <sub>(max)</sub> (ng/mL)	6.6	NA	NA	NA

NA = samples not available due to deaths of the animals

Pharmacodynamics:

Text Table 2. Mean 20S Proteasome Specific Activity (% gestation Day 6 predose)

PS-341 (mg/kg/day)	Predose	30 min	1 hr	6 hr	24 hr
<b>Gestation Day 6</b>					
0	100	64	73	32	61
0.05	100	21	30	40	61
0.10	100	14	18	26	37
0.15	100	13	16	31	41
0.20	100	11	16	28	35
<b>Gestation Day 17</b>					
0	80	105	78	80	80
0.05	50	15	21	34	50
0.10	NA	10	3	18	30
0.15	NA	NA	NA	NA	NA
0.20	NA	NA	NA	NA	NA

NA= Data not available due to mortality or termination of group.

**Summary of individual study findings in the rat:**

Results of this range-finding study in the rat showed that three of the four doses administered on gestational day (GD) 6 through 17, inclusive, were too high, as they were maternally lethal. No dams in the HD (0.20 mg/kg/day) or MD2 (0.15 mg/kg/day) group survived to necropsy. Only one MD1 (0.10 mg/kg/day) dam survived, but total resorption was noted in this female. The only viable dose tested, LD (0.05 mg/kg/day), was not maternal, embryo, or fetal toxic. Gross pathology changes were seen in the MD1 dams that were euthanized prior to study completion and the one surviving MD1 dam. The MD2 and HD dams showed no similar macroscopic changes, though this was most likely due to the lethality of the PS-341 shortly after treatment began.

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On GD 6, a non-linear increase was seen in both the AUC<sub>(0-24)</sub> and the C<sub>max</sub> of PS-341 over the four doses tested. Toxicokinetic data from the LD group on day 17 of gestation showed an AUC<sub>(0-24)</sub> of 99 hr·ng/mL, compared to one of 21 hr·ng/mL seen on the first day of drug exposure. Although this was the only group with data from two time points, it appears that accumulation of the drug occurs over the course of treatment, as both the AUC and C<sub>max</sub> increased 3-4 fold. These results are consistent with other pharmacokinetic data obtained in rat. The number of time points sampled in this study are very small, though the similarity with other rat study results adds weight to these estimates of AUC and C<sub>max</sub> being adequate representations of the pharmacokinetics of PS-341 in the pregnant rat.

Pharmacodynamic data showed that maximum 20S proteasome activity inhibition was seen at the 30-minute post-dose time point in all doses, with 79-89% inhibition seen on GD 6. The 24-hr time point saw a trend toward recovery, with inhibition ranging from 39-65% at this time point. The predose inhibition level on GD 17 with the LD group was 50%, indicating that inhibition from previous dosing with PS-341 was still evident. This inhibition level was, in fact, lower than that seen in this dose group at the 24hr time point on GD 6. This would be most likely indicative of the drug accumulation that toxicokinetic parameters show is occurring. The inhibition seen over the 24 hour evaluation on GD 17 was comparable to that seen on GD 6 with a maximum inhibition seen at 30 minutes (85%) and incomplete recovery seen at the 24-hr point, with inhibition levels similar to the pre-dose values (50%). One MD1 sample was taken at each time point on GD17, with no sample taken at the pre-dose point. Inhibition was seen at each time, ranging from 97% at 60 minutes to 70% at 24 hours. As these are all single samples, they are informative but not evaluable for significance. Of note also is the variability seen in the control group. At the 6-hr time point on GD 6, 68% inhibition of 20S proteasome activity was seen in the samples taken from 3 rats that were never dosed with PS-341. This variability may likely be due to inaccurate methodologies used to examine this pharmacodynamic activity. The variability seen in this group on GD 6 is not as prominent on GD 17, though 22% inhibition is still seen in the control group at 60 minutes on that day.

No external malformations were noted in the LD group, the only dose with viable fetuses. No information could be obtained from the other 3 doses tested, due to the maternal lethality. Embryo lethality is evidenced by the one surviving MD1 dams total resorption of the litter.

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**Study title:** An intravenous injection teratology study of PS-341 in the Sprague-Dawley rat.

**Key study findings:**

- Slight maternal toxicity at HD (0.075 mg/kg/day), as evidenced by body weight and food consumption decreases.
- No teratogenic effects seen on skeletal or visceral development.
- No effects seen on embryo-fetal toxicity or lethality.

**Study no.:** Project No. 98173  
**Volume #, and page #:** Module 4: 4.2.3.5.2.2  
**Conducting laboratory and location:**    
**Date of study initiation:** 26 March 2002  
**GLP compliance:** Compliance included and signed  
**QA reports:** Yes (X) no ( )  
**Drug, lot #, and % purity:** PS-341, lot# D2-1-1, purity not given  
**Formulation/vehicle:** D-Mannitol in 0.9% NaCl

**Methods:**

**Species/strain:** Rat/Sprague Dawley CD (CrI:CD@[SD-7784]IGS BR)  
**Doses employed:** Vehicle control = 0  
 LD = 0.025 mg/kg/day  
 MD = 0.050  
 HD = 0.075  
**Route of administration:** IV bolus injection; variable volume  
**Study design:** Segment II Study  
**Females** – Dosed once daily gestational day (GD) 6-17  
 Euthanized on GD 20  
**Males** – Not dosed  
**Number/sex/group:** 22 ♀/group  
**Parameters and endpoints evaluated:** **Females**  
 Clinical observations, maternal body weights and food consumption, # corpora lutea, # of implantation sites, # of pre-implantation losses, # of resorptions, individual weights and sex of fetuses, external observations of fetuses and visceral and skeletal examinations of fetuses.

**Results:**

**Maternal toxicity:**  
**Mortality:** No mortality  
**Clinical signs:** 3/22 HD dams – yellow staining at urogenital area  
 1/22 HD dam – decreased fecal output, thin, dehydrated,  
 and ↓ activity  
**Body weight:** HD – GD 6-12 body weights ↓ 5-6% compared to controls

GD 12-15 – ↑ weight gains bringing actual body weights comparable to controls for remainder of gestation (GD 15-20).

Food consumption: HD – GD 6-9 – 25 % ↓ in food eaten compared to controls  
 HD – GD 9-12 – 12% ↓ in food eaten compared to control

In-life observations: No treatment-related effects on pregnancy rate.

Terminal and necroscopic evaluations:

Dams: No treatment-related effects on gross pathology effects.  
 No treatment-related effects on # of corpora lutea.  
 No treatment-related effects on # of implantations.  
 No treatment-related effects on resorptions.  
 No treatment-related effects on pre-implantation loss.  
 No treatment-related effects on post-implantation loss.

Offspring: No treatment-related effects of fetal weights.  
 No treatment-related effects on number of live/dead fetuses.

**Major malformations**

No significant treatment-related effects on major malformations. Incidences seen were isolated and well within historical control.

**Minor external and visceral anomalies**

LD fetuses – Dilated ureters (2/153 fetuses; 2/21 litters)  
 Reduction of renal papillae (1/153 fetuses; 1/21 litters)

All within historical controls

**Minor skeletal anomalies**

MD and HD – significantly lower incidence of fetuses with minor skeletal anomalies

Hyoid bone reduced ossification:

MD = 51% ↓ in fetuses affected

HD = 61% ↓ in fetuses affected

Supraoccipital bone reduced ossification:

MD = 79% ↓ in litters and 83% ↓ in fetuses affected

Interparietal bone reduced ossification:

HD = 62% ↓ in litters and 70% ↓ in fetuses affected

**Common skeletal variants**

No treatment-related effects.

**Summary of individual study findings in the rat:**

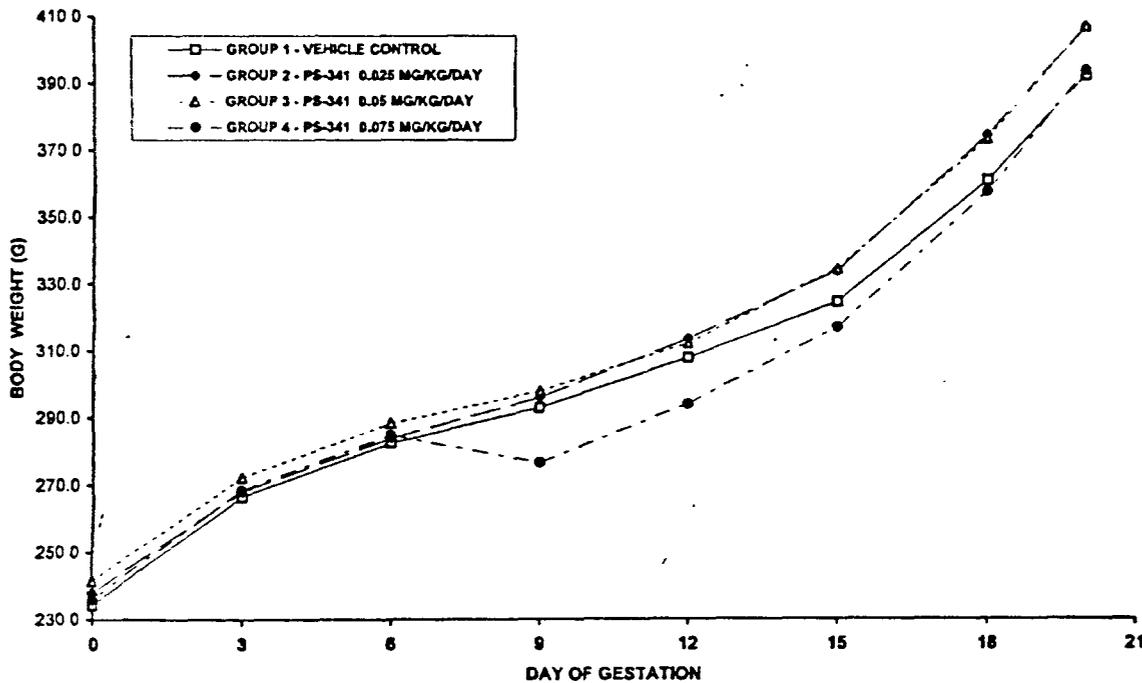
The doses used for the complete teratology study of PS-341 in rats were adjusted down based on the lethality seen in the range-finding study. No lethality or significant clinical signs were seen in the definitive study. Food consumption by the HD dams (0.075 mg/kg/day) was

decreased from GD6-12. HD dams lost weight in the early stages of drug administration, GD 6-9. While the control group averaged a gain of 10.4 g, the HD rats lost 8.2 g. Because of this, the HD dams body weights were significantly lower until GD 12. The HD group then rebounded with larger increases in weight gains from GD 12 until 15 than the control dams, resulting in comparable final body weights when HD dams were compared to controls. The lack of difference between final body weights among groups in this study does not impact the credibility of the findings. Ideally, studies designed to show teratogenic effects of a compound should show an impact on the maternal weight gain during the time of dosing, organogenesis. Rebounding after drug administration has ended is not uncommon and does not impact the validity of the study. Effect of the maternal drug administration on maternal weight gain during organogenesis is a more sensitive gauge of toxicity in Segment II studies than overall weight gain. The rebound weight gain began during drug administration, which may have indicated some developing tolerance to the drug. The corrected body weight gains at the end of the study differed, with 33.6g gain seen in control animals and a 30.4g gain seen in the HD dams. Statistics were not provided, but this is likely not significant, as the HD gains are affected by one rat who actually lost 56g in the corrected body weight from GD 6-20. As the data show that the HD rats rebounded after early weight loss in the beginning of drug administration, this is to be expected. The following graph illustrates these changes.

FIGURE NO. 1

GROUP MEAN BODY WEIGHTS (G) OF PREGNANT FEMALES

PROJECT NO. 98173



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Examination of the fetuses exposed to PS-341 during organogenesis showed no significant effects of the drug on skeletal and visceral development. The few changes seen, were within historical control ranges or comparable to the concurrent control group. Incidences of minor skeletal anomalies were actually lower in the MD (0.05 mg/kg/day) and HD litters compared to control litters. These types of variations are not uncommon and the differences in incidences of reduced ossification do not impact the offspring adversely. Why this variation was seen more in the lower dose and control groups, than in the two highest PS-341 doses, is not clear. One possibility could be the schedule in which dams were euthanized and the fetuses removed for examination. Ossification occurs rapidly during the last 48 hours of gestation. If the litters were euthanized in a systematic order with control groups taken first, followed by increasing doses, then the higher dose group litters may have had enough extra gestation time to allow for a noticeable change in this parameter. Regardless, this change is of little clinical significance. No embryo-fetal toxicity or lethality was seen at any dose, even when indications of maternal toxicity were present.

The doses used were based on the dose-range finding study, which had excessive maternal toxicity and lethality. The highest dose used in the definitive study was 0.025 mg/kg/day lower than the range finding study dose of 0.10 mg/kg/day that was maternally lethal in all but one rat. The highest dose used in this definitive study, 0.075 mg/kg/day, may not have been the highest dose possible that would enable viable, evaluable litters. But given the steep toxicity curve that has been shown in the range finding study, as well as other rat toxicology studies, this dose seems appropriate for the HD in this study. The rat toxicology studies utilized an intermittent dosing schedule. The dosing schedule used for Segment II reproductive studies of 12 days continuous dosing, would most likely lead to more toxicity than seen in the toxicology studies, especially given the drug accumulation that has been noted in the rat.

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**Study title:** An intravenous range-finding teratology study of PS-341 in the New Zealand white rabbit.

**Key study findings:**

- No evidence of embryo-fetal lethality or toxicity at the doses tested when administered from gestational day (GD) 7 –19, inclusive.
- No treatment-related maternal toxicity.
- Toxicokinetic data show non-linear increases in  $AUC_{(0-24)}$  and  $C_{max}$  with increased doses of PS-341.
- Pharmacodynamic data show 80-90% inhibition for PS-341 doses 30 minutes after the first drug administration on GD 6 in the LD group.

**Study no.:** Project Number 98174

**Volume #, and page #:** Module 4; 4.2.3.5.2.3

**Conducting laboratory and location:** [ ]

**Date of study initiation:** 25 June 2002

**GLP compliance:** Not conducted in full compliance of GLP  
Sponsor claims deviations documented in raw data and did not affect results or interpretation.

**QA reports:** yes ( ) no (X )

**Drug, lot #, and % purity:** PS-341; #D2-1-1; purity not given

**Formulation/vehicle:** D-Mannitol in 0.9% NaCl

**Methods:**

**Species/strain:** Rabbit/New Zealand White

**Doses employed:** Vehicle control = 0  
LD = 0.01 mg/kg/day  
MD1 = 0.025  
MD2 = 0.04  
HD = 0.05

**Route of administration:** IV bolus injection; variable volume

**Study design:** Segment II Study  
**Females** – dosed daily during gestation – from gestational days (GD) 7-19  
Main study animals euthanized on GD 29  
Satellite animals euthanized on GD 19  
**Males** – not dosed

**Number/sex/group:** 5 ♀/group  
3 ♀ for control and HD satellite groups for toxicokinetic and pharmacodynamic evaluations

Parameters and endpoints evaluated: **Females**  
 Clinical observations, maternal body weights and food consumption, # corpora lutea/ovary, weight of uterus + contents, # and position of live and dead fetuses, # and position of early and late embryo/fetal losses, individual weights and sex of live fetuses, external observations of live fetuses.

Toxicokinetics: Blood samples pre-treatment and 30 min, 1, 6 and 24 hr post-treatment on GD 7 and 19. Satellite groups for control and HD also bled on GD 19 at 5, 10, 15, 30 and 60 minutes post-treatment.

Pharmacodynamics: Blood samples obtained for toxicokinetics also used for determination of 20S proteasome activity.

**Results:**

Mortality: No treatment-related deaths

Clinical signs: No treatment-related clinical signs

Body weight: No treatment-related changes in body weights, body weight gains.

Food consumption: No treatment-related changes in food consumption.

In-life observations: No treatment-related effects on:  
 Pregnancy rate  
 Number of live fetuses  
 Sex ratio of fetuses  
 Fetal body weights

Terminal and necroscopic evaluations:  
 Dams: **No treatment-related effects on:**  
 Number of corpora lutea  
 Number of implantation sites  
 Gravid uterine weights  
 Number of resorptions  
     Control – 1/8 does (includes TK satellite rabbits)– 1 resorption  
     LD – 1/5 does – 1 resorption  
     MD1 – 3/4 does – 2 does 1 resorption each, 1 doe 2 resorptions  
     MD2 – 1/5 does – 1 resorption  
     HD 1/8 does (including TK does) – 5 resorptions and lower gravid uterine weight  
     HD 1/8 does (including TK does)- aborted

Offspring:  
 Gross pathology  
 No treatment-related external fetal effects

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**Toxicokinetics:**

Text Table 1. Mean Values for Gestation Day 7 and Day 19 Toxicokinetic Parameters

		PS-341 (mg/kg/day)				
		0.010	0.025	0.040	0.050	0.050*
<b>Gestation Day 7</b>						
AUC <sub>(0-24)</sub> (hr*ng/mL)	ID	9.1	19.9	6.9	NS	
C <sub>(max)</sub> (ng/mL)	ID	1.27	1.91	1.89	NS	
<b>Gestation Day 19</b>						
AUC <sub>(0-24)</sub> (hr*ng/mL)	30.8	98.2	131.0	114.0	120.0	
C <sub>(max)</sub> (ng/mL)	1.62	6.16	6.96	6.51	22.60	

\*Additional animals for assessment of 5 min to 1 hr postdose plasma levels.  
ID=insufficient data to calculate.  
NS=samples not taken.

**Pharmacodynamics:**

Text Table 2. Gestation Day 7 and Day 19 Mean 20S Proteasome Specific Activity

		Mean 20S Proteasome Specific Activity (%)*				
PS-341 (mg/kg/day)	Pre-dose	30 min	1 hr	6 hr	24 hr	
<b>Gestation Day 7</b>						
0	100	104	95	101	99	
0.010	100	82	82	94	90	
0.025	100	65	70	85	94	
0.040	100	50	48	71	91	
0.050	100	47	53	76	90	
<b>Gestation Day 19</b>						
0	98	97	101	89	97	
0.010	82	65	66	76	91	
0.025	72	35	39	52	70	
0.040	61	28	32	45	70	
0.050	63	25	26	35	63	

\*The specific activities at pre-dose gestation Day 7 for each dosage group were used to calculate percentages.  
Values represent the average of five animals per time point.

**Summary of individual study findings in the rabbit:**

No treatment-related maternal toxicity was noted. A HD (0.50 mg/kg/day) female aborted the litter during GD 20-23. This could not be linked to drug treatment as it occurred in only one doe and rabbits historically show a 5% abortion rate. Variability in resorptions was seen, all the groups showing some incidence of resorptions. One or two resorptions were seen in each dose group, with 5 resorptions seen in one HD litter. The MD1 (0.025 mg/kg/day) dose group is the only group with more than one litter with resorptions. The lack of a clear dose-response effect on resorptions precludes determining any drug contribution to this effect. No effects were seen on the fetal parameters that were measured, which included body weights and external malformations. The doses tested did not appear to cause any significant effects on embryo-fetal lethality or toxicity.

Following the first exposure to drug on GD 7, toxicokinetic data show near-linear increases in AUC and C<sub>max</sub> from the MD1 to the MD2 (0.40 mg/kg/day) dose level. This same pattern was not seen with the HD animals. The AUC in the HD rabbits was actually lower than the AUC in the MD1 group. There was insufficient data to calculate these parameters for the LD (0.10 mg/kg/day) group. Following 13 consecutive days of PS-341 treatment, on GD 19, toxicokinetic parameters again showed increased AUC and C<sub>max</sub> over the LD, MD1 and MD2 groups, with the AUC showing linear increases as the dose increased. At this time point, as was seen on GD 7, the HD group does not follow the same pattern both

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AUC and  $C_{max}$  at the HD level are lower than that of the MD2 rabbits. As was seen in the rat, the toxicokinetic parameters showed increases from the first day of drug administration to the last day, indicative of drug accumulation. Similar to the rat study, the number of time points sampled for toxicokinetics was smaller than is normally accepted. A minimum of eight time points would be more appropriate. With the fewer time points, the toxicokinetics from this study should be considered an estimation of the AUC and  $C_{max}$ .

Pharmacodynamic data in the rabbit do not show the same problematic proteasome inhibition in the control group that was seen in the rat study. As in the rat, maximum inhibition was noted at the 30-min post-treatment time point, 18-53% on GD 7 and 35-75% on GD 19. On the first day of PS-341 treatment, inhibition was not seen at the 24-hr post-treatment time point, showing recovery within 24 hours. This differs from the rat, where inhibition was still evident at the 24-hr time point on the first day of drug administration. Similar to the rat though, inhibition was seen at the predose time point on GD 19, which could be explained by the drug accumulation that is apparent from the toxicokinetic data. Unlike on GD 7, inhibition was still evident at the 24-hr time point. No inhibition was seen in the limited fetal blood that was obtained at the 1-hr time point on GD 19. This result is of little scientific significance as there was insufficient blood from any earlier time points.

The doses used in this study were determined by a previous toxicology study conducted in non-pregnant New Zealand white rabbits (Project # 57359; Module 4.2.3.2.6). In this general toxicology study, 3 rabbits/dose were administered PS-341 at doses of 0.025, 0.05, 0.075 and 0.1 mg/kg/day intravenously for 13 consecutive days. Mortality was seen in the two highest doses. Two rabbits were found dead and the third euthanized in a moribund condition in the HD (0.1 mg/kg/day) group. The MD2 group (0.075 mg/kg/day) had one rabbit that died and the other two euthanized on Day 7 of dosing. Because of this, both these doses were considered too high for further study and the MD1 dose of 0.05 mg/kg/day was used as the highest dose tested in the dose range-finding study in pregnant New Zealand white rabbits.

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**Study title:** Intravenous teratology study of PS-341 in the New Zealand white rabbit.

**Key study findings:**

- No significant teratogenic effects of PS-341 seen in the New Zealand white rabbit.
- Embryo lethality was seen, as evidenced by decrease live fetuses and increased resorptions seen at the highest dose tested, .050 mg/kg/day (AUC<sub>(0-24)</sub> of 114.0 hr·ng/mL by day 19 of gestation – see previous study).

**Study no.:** Project Number 98175  
**Volume #, and page #:** Module 4; 4.2.3.5.2.4  
**Conducting laboratory and location:** [ ]  
**Date of study initiation:** 5 August 2002  
**GLP compliance:** Letter included and signed  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** PS-341; #D2-1-1; purity not given  
**Formulation/vehicle:** D-Mannitol in 0.9% NaCl

**Methods:**

**Species/strain:** Rabbit/New Zealand White  
**Doses employed:** 0, 0.01, 0.025, 0.05 mg/kg/day  
**Route of administration:** IV bolus injection; variable volume  
**Study design:** Segment II Study  
**Females** – dosed daily during gestation – from gestational days (GD) 7-19  
**Males** – not dosed  
**Number/sex/group:** 22 ♀/group  
**Parameters and endpoints evaluated:** **Females**  
 Clinical observations, maternal body weights and food consumption, # corpora lutea, # of implantation sites, # of pre-implantation losses, # of resorptions, individual weights and sex of fetuses, external observations of fetuses and visceral and skeletal examinations of fetuses.

**Results:**

**Mortality:** One HD doe found dead on GD 17.  
**Clinical signs:** 19% (4/21) HD does showed signs of abortion  
**Body weight:** Significant body weight gain effect on HD does  
 ↓ 27% compared to controls  
 Most likely due to 4 does that showed signs of abortion – did not gain weight between GD 7 – 20.  
**Food consumption:** Slight decrease in food consumption, GD 9 –17, HD does compared to controls.

In-life observations: No treatment-related effects on pregnancy rate  
Terminal and necroscopic evaluations:

Dams: No treatment-related effects on # of corpora lutea, implantation sites or pre-implantation losses.

Late resorptions –  
HD 10 late resorptions seen in 6/16 (37.5%) does compared to control of 2 late resorptions seen in 1/22 (4.5%) litters. This is a 400% ↑ in # of late resorptions and 733% ↑ in litters affected

Total resorptions –  
: HD 18 total resorptions seen in 9/16 (56%) does compared to control of 4 total resorptions seen in 3/22 (13.6%) litters. This is a 350% ↑ in # of total resorptions and 311% ↑ in litters affected

Post-implantation loss – HD mean of 14%, control mean of 2.1% = ↑ 567% compared to control

Gravid uterine weight – HD ↓ 22% compared to control, not significant

Gross pathology – HD 2/22 does with dark foci in stomach  
MD 2/22 does with dark foci in stomach  
HD 2/22 does with thickening of wall of pyloric region

Offspring:

Number of live fetuses –  
HD mean of 6.3 fetuses ↓76% compared to control mean of 8.3 live fetuses per litter

Fetal weights – HD fetal weights significantly ↓ compared to control – Combined ♂/♀ weights – ↓ 8%

Major malformations – No treatment effects  
Minor external anomalies – No treatment effects  
Minor visceral anomalies – Significant increase in fetuses with minor anomalies, due to HD fetuses with absence of accessory lobe of the lungs. Occurred in 7 fetuses from 2 different litters. The litter incidence was within historical control values.

Minor skeletal anomalies – Significant increase in HD fetuses and litters with incidence of ossification centers on the 7<sup>th</sup> cervical vertebrae compared to the control group. The percentage was, however, comparable to historical controls for both litters and fetuses.

Common skeletal variants – No treatment effects

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**Summary of individual study findings in the rabbit:**

The doses used in this definitive study were taken directly from the dose range-finding study. The lack of a clear definitive maternal toxicity in that study would indicate that a clearer picture may have been obtained if a higher dose, clearly maternally toxic or lethal, had been tested. However, unlike in the range-finding study, embryo-fetal lethality or toxicity was demonstrated with the highest dose tested, 0.05 mg/kg/day when administered to pregnant rabbits during gestation, from days 7-19. While no toxicokinetics data were obtained in this study, this dose corresponds to an AUC<sub>(0-24)</sub> of 114 hr·ng/mL by day 19 of gestation, based on the results of the dose range-finding study. At this dose level, embryo lethality was noted by the increase in late resorptions and post-implantation loss seen when compared to control. There was a significant decrease in the number of live offspring in the HD group. Fetal toxicity was evident in the decreased fetal weights, using litter size as a covariate, in the HD group.

Lethality was seen in 1/22 HD (0.05 mg/kg/day) rabbits. The rabbit was cold to the touch, with decreased activity and soft/liquid feces. The cause of death, however, was undetermined. Slight maternal toxicity was evident in the decreased food consumption seen with the HD rabbits. The minor macroscopic findings in the stomachs of does from the MD (0.025 mg/kg/day) and HD group were also indicative of maternal toxicity. While there were decreases in the body weight gains of these HD rabbits, this was most evident post-administration of PS-341 and attributable more to the aborted litters than to direct maternal toxicity from drug administration. When body weight gains are corrected for the gravid uterus, the HD rabbits do show a decrease in body weight of a mean of .009 kg as opposed to a body weight gain of .019 kg in the control. While the effects on corrected body weight are most likely indicative of maternal toxicity at this dose, it is not as definitive without the statistical significance or as evidence of weight loss during the drug administration period would have been. Because of this, maternal toxicity alone may not explain the effects seen on resorptions, post-implantation loss, live offspring, and fetal weights. The graph below illustrates the maternal body weight effects and illustrates how the drug effects on weight gain occurred after drug administration had ceased. This is a time where rapid growth of the fetuses would be represented in the body weight gain of the does.

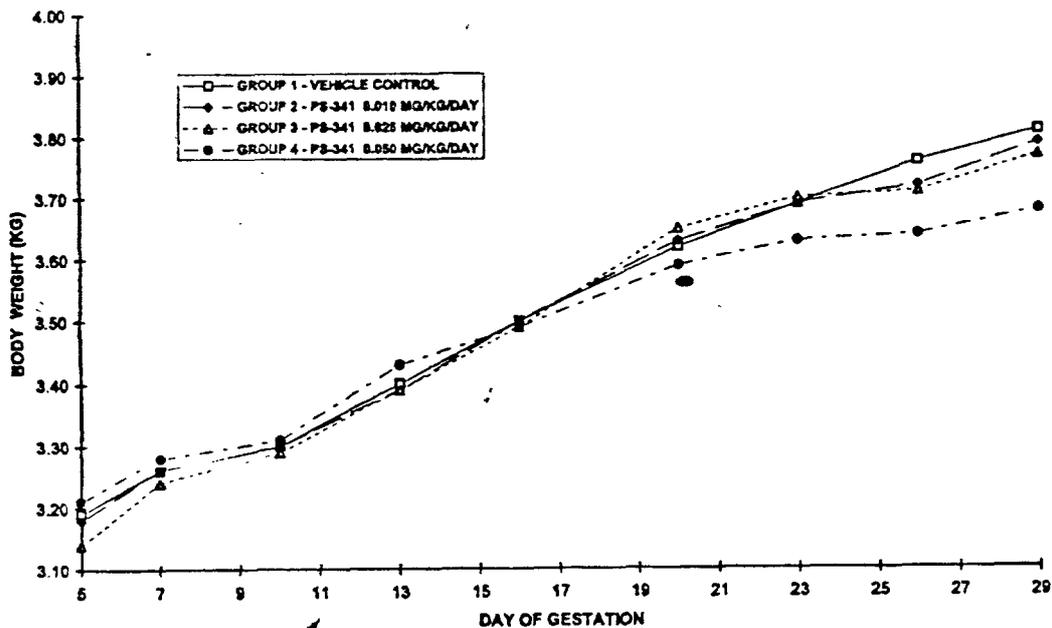
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FIGURE NO. 1

GROUP MEAN BODY WEIGHTS (KG) OF PREGNANT FEMALES

PROJECT NO. 98175



The teratology results indicate no clear treatment-related effects on skeletal and visceral malformations, anomalies or variants. While an increase was seen at the HD level in fetuses with absence of accessory lobe of the lungs, the litter incidence was within the historical control range. Also within historical control range was the overall incidence of litters and fetuses with minor skeletal anomalies.

The lower doses tested, 0.10 and 0.25 mg/kg/day, with corresponding AUC<sub>(0-24)</sub> levels of 31 hr·ng/mL for the LD and 98 hr·ng/mL for the MD groups, by day 19 of gestation, had no significant maternal or embryo-fetal effects.

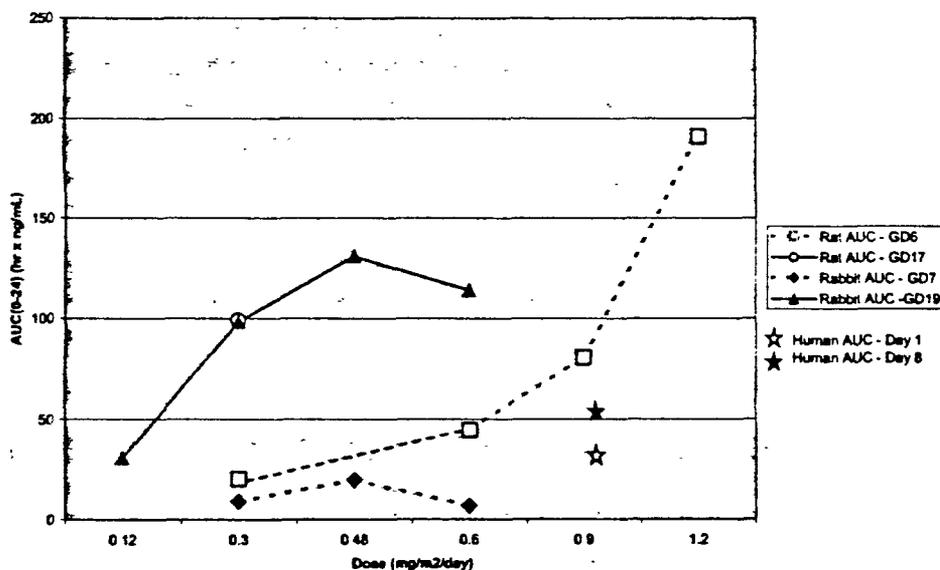
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**Reproductive toxicology summary:**

Teratological effects of PS-341 were examined in both the rat and rabbit. In neither species were there significant increases in major skeletal or visceral malformations due to the in utero exposure to PS-341. Minor skeletal and visceral anomalies were seen in the rabbit litters, but these incidences were within the historical control.

Toxicokinetic parameters in both species, although from a minimal number of samples, showed drug accumulation over the course of the study. This had previously been demonstrated with the rat in toxicology studies with intermittent doses, so it was an expected result. The results from the rabbit reproductive study are the only data for this species. The toxicokinetics in the rabbit show a plateau in the parameters that has not been seen in the rat. The AUC and  $C_{max}$  in the rabbit also appear to be lower, at proportional doses, than what is seen in the rat. The lack of surviving rats for the final day of sampling makes it more difficult to come to firm conclusions. Samples from the first day of drug administration do show that the AUC and  $C_{max}$  in the rat are higher than that seen in the rabbit. While little toxicity was seen in the dose range-finding rabbit study, as no lethality and very minimal maternal toxicity were noted, the toxicokinetic data indicate that higher doses may not have led to any higher blood levels of PS-341. In addition, a toxicology study in non-pregnant also indicates that a slight increase in dose,

Toxicokinetic Parameters of PS-341 in the Rat and Rabbit During Gestation

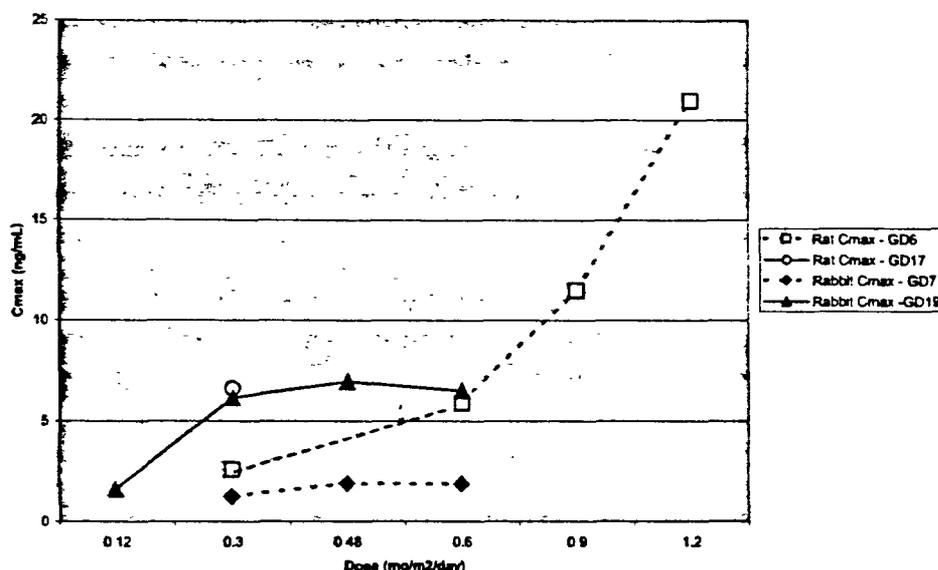


from 0.6 to 0.9 mg/m<sup>2</sup>/day, led to lethality or moribund animals.

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Toxicokinetic Parameters of PS-341 in the Rat and Rabbit During Gestation



Clinical data from an intermittent dose of 1.0 mg/m<sup>2</sup>/day show an AUC of 30.1 hr·ng/mL on Day 1 and 54.0 hr·ng/mL on Day 8. Based on this, the rabbit blood levels by the end of dosing are above the blood levels seen in the humans in the two highest doses tested in the definitive teratology study.

Proteasome inhibition was seen in both the rat and rabbit following PS-341 administration. In both species, maximum inhibition occurred at the 30-min post-drug time point. In both species, inhibition was evident at the predose time point on the final day of drug administration, evidence of the drug accumulation that occurred in both species. Proteasome inhibition appears to be greater in the rat than it does in the rabbit. For the first day of treatment, this may be attributable to the lower AUC and C<sub>max</sub> seen in the rabbit. Without additional rat data, it is difficult to make firm conclusions about differences in toxicokinetics and pharmacodynamics between the two species. Perhaps the lack of the extreme toxicity profile seen in the pregnant rabbits in the dose range-finding study could be, in part, due to the lower 20S proteasome inhibition that is seen in this species.

Embryo-fetal toxicity and lethality were seen in the rabbit. These effects included increases in post-implantation loss, total resorptions and late resorptions. The live fetuses from these litters, despite being from smaller litters, were also smaller in weight than the control group. This was seen at the highest dose, 0.05 mg/kg/day, which is equivalent to 0.6 mg/m<sup>2</sup>/day and approximately half of the clinical dose of 1.3 mg/m<sup>2</sup>. The difference in schedules make comparison between the two difficult, as the rabbit was dosed daily for 13 days, as opposed to the intermittent schedule used clinically. For this reason, cross-species comparison to humans will be based on dose administered, corrected for body surface area.

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**Reproductive toxicology conclusions:**

Adequate doses were tested in the rat definitive study. While higher doses may have been tolerated in the rabbit, toxicokinetic data indicate that adequate exposure levels were achieved in this study and may not have been able to be increased with higher doses. The lack of any other rabbit studies with this compound make it difficult to know if higher doses would have been tolerated without significant lethality. Other species have shown very steep dose curves for toxicity, with small increases in dose leading to large increases in toxicity. Without additional studies in the rabbit, it is unknown if this would have been seen in that species as well.

Litter parameters such as resorptions, number of live fetuses, and fetal weights, were not impacted by PS-341 treatment in the definitive rat study. These parameters were adversely affected in the rabbit. The highest dose tested, equivalent to 0.6 mg/m<sup>2</sup>/day, led to decreases in the number of live fetuses and the combined fetal weights of both sexes. It also impacted the number of resorptions, late and total, and post-implantation loss. While maternal toxicity is certainly evident at this dose group, the toxicities are mostly minor, such as decreased food consumption and small decreases in weight gain. Clinically, these are not toxicities that would lead to drug cessation. That embryo-fetal lethality and toxicity was noted at doses with minimal maternal toxicity has important clinical ramifications. In humans, treatment with PS-341 (Velcade™) could impact the ability to maintain viable pregnancies. It is important that female patients are aware of this potential and will take precautions to avoid pregnancy.

Overt teratology has not been seen in rat or rabbit reproductive studies. Embryo-fetal toxicity and lethality has been seen in the rabbit at doses that although maternally toxic, were still well tolerated by the rabbits. This is important information clinically and should be included in the label.

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**VIII. SPECIAL TOXICOLOGY STUDIES:**

Reviewed by Lilliam A. Rosario, Ph.D.

Study title: A PERIVASCULAR/ INTRAVASCULAR, SUBCUTANEOUS, AND INTRAMUSCULAR IRRITATION STUDY WITH PS-341 IN MALE NEW ZEALAND WHITE RABBITS

**Key study findings:**

- The single injection of PS-341 in the male New Zealand white rabbit induced microscopic changes at the perivenous and intramuscular injection sites of 6/6 and 1/6 animals, respectively.
- No test article-related changes were observed following intravenous and subcutaneous administration of PS-341 in the rabbit.

Study no: 57360

Volume #, and page #: Module 4

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 26 February 2002

GLP compliance: Yes

QA reports: yes (x) no ( ):

Drug, lot #, radiolabel, and % purity: PS-341/mannitol Lot # D2-1-1 \_\_\_\_\_

Formulation/vehicle: Reconstituted by injecting 3.5 mL of 0.9% Sodium Chloride for Injection

**Methods:**

**Dosing:**

Group No	Route of administration	Dose level (mg/kg)	Dose concentration (mg/mL)	Animal numbers**
1*	D-mannitol	1	10	101-106
2*†	PV (PS-341)	0.1	1	201-206
3*†	IV (PS-341)	0.1	1	301-306
4*†	IM (PS-341)	0.1	1	401-406
5*†	SC (PS-341)	0.1	1	501-506

\*Control and test material was administered into the left and right side of the body, respectively.

†Control was 0.9% Sodium Chloride for Injection U.S.P.

‡Control animals were dosed PV, IV, IM, and SC with 0.9% Sodium Chloride for Injection, U.S.P. on the left side of the body, and with D-mannitol on the right side of the body. IV doses were administered prior to PV doses

\*\*3 males were sacrificed at 24 hours post-dose, 3 males were sacrificed at 72 hours post-dose.

PV – perivenous, SC – subcutaneous; IV – intravenous; IM – intramuscular

**Observations and times:**

Clinical Examinations: All animals were examined twice daily for mortality and signs of ill health or reaction to treatment.

Draize Examinations: Evaluation of injection sites for erythema/eschar and edema were conducted on Day 1 at predose, immediately following dosing and at approximately 1, 2, 4 and 6 hours post-injection and twice daily (AM, PM, circa 6 hours apart) thereafter.

Body Weight: Pretreatment period for randomization purposes, predose for dose volume calculations, and prior to necropsy for anesthetic dose calculation purposes only. These data were retained on file but not reported.

Gross Pathology: The necropsy consisted of an external examination of the injection sites.

Tissue Preservation: On completion of the necropsy of each animal, the animal identification, abnormal tissues and injection sites (ear vein, ear artery, perivenous space and subcutaneous space) were retained in neutral buffered 10% formalin.

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Histopathology: The negative control, vehicle control, and test article injection sites of all animals were examined histopathologically.

**Results:**

Mortality: There was no mortality during this study.

Evaluation of skin reactions:

Perivenous injection sites:

There was no difference in the incidence, severity or duration of erythema or edema when comparing the perivenous administration of saline, D-mannitol or PS-341 at a dose level of 1.2 mg<sup>2</sup>.

Intravenous Injection:

There was no difference in the incidence, severity or duration of erythema or edema when comparing the perivenous administration of saline, D-mannitol or PS-341 at a dose level of 1.2 mg<sup>2</sup>.

Intramuscular Injection:

When compared to the contralateral saline control, a slight increase in the incidence of erythema was noted in two animals (numbers 403 and 406) treated with an intramuscular injection of PS-341 at 1.2 mg<sup>2</sup>. In addition, slight edema was noted in animal 406 from Day 2 of the observation period.

Subcutaneous Injection:

When compared to the contralateral saline control, following subcutaneous administration of PS-341, no difference in the incidence, severity or duration of erythema or edema was observed. Slight erythema was noted at the PS-341 injection site in one animal (number 506), with slight edema also noted in this animal at the saline injection site.

Macroscopic Findings: The macroscopic findings observed at the injection sites of control and treated animals were interpreted as the result of the experimental injection procedures. Other changes noted in the lung and thymus were consistent with the normal spontaneous changes observed in animals of this species.

## Microscopic Findings:

Perivenous injection sites:

- Test article-related findings were present in the PS-341 perivenous injection sites of 6/6 animals.
- These findings were characterized by the presence of perivascular inflammation and edema ranging from minimal to moderate.
- The inflammation was primarily heterophilic (acute) at 24 hours post-injection and became mononuclear (chronic) at 72 hours post-injection. At 24 hours post-injection, the PS-341 injection sites of 3/3 animals demonstrated necrosis of isolated keratinocytes in the stratum spinosum. This finding was confined to the site of inflammation and edema. The change was reversible considering that at 72 hours, the epidermis was unremarkable. However, at 72 hours post-injection, 2/3 animals demonstrated minimal epithelial parakeratosis. This finding was interpreted to be the result of the epithelial necrosis observed at 24 hours post-injection.
- Other changes observed in the perivenous injection sites of the negative control, vehicle control, and test article were interpreted to be the result of the experimental procedures.

Intramuscular injection sites:

- Test article-related findings were present in the PS-341 intramuscular injection site of 1/3 animals at 24 hours post-injection.
- Locally extensive moderate degeneration and necrosis of myofibers were present. Myofibers were swollen, eosinophilic and fragmented with a moderate heterophilic (acute) inflammation.
- The pathologist states that "Even though there is a low incidence of the lesion (1/3 animals at 24 hours post-injection), the nature of the change and the severity is suggestive of a test article-related change".

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- Other changes observed in the intramuscular injection sites of the negative control, vehicle control, and test article were interpreted to be the result of the experimental procedures. Changes included degeneration and necrosis of few myofibers with associated minimal to slight mononuclear inflammation. Also present were hemorrhages within the fascia with associated inflammation.

Intravenous and subcutaneous injection sites:

- No vehicle control or test article related microscopic changes were observed. The changes seen were infrequent and interpreted as the result of the experimental procedures.

**Summary of individual study findings:**

Administration of 1.2 mg/m<sup>2</sup> PS-341 to male New Zealand white rabbits as a single intravenous, perivenous, intramuscular or subcutaneous injection was tolerated. PS-341 treatment resulted in a slight increase in incidence of erythema and/or edema following intravenous and intramuscular administration as compared to saline with recovery by 72 hrs post injection. However, microscopic changes were observed following perivenous (6/6 rabbits) and intramuscular (1/3) injection. Minimal to moderate perivascular inflammation and edema were observed. At 24 hours post-injection, the PS-341 injection sites of 3/3 animals demonstrated necrosis of isolated keratinocytes in the stratum spinosum. At 72 hours post-injection, 2/3 animals demonstrated minimal epithelial parakeratosis. After intramuscular administration, locally extensive moderate degeneration and necrosis of myofibers were present. Myofibers were swollen, eosinophilic and fragmented with a moderate heterophilic (acute) inflammation. No difference in erythema or edema was noted between 10 mg/mL mannitol and saline, following perivenous, intravenous, or subcutaneous administration. Changes after intramuscular administration of mannitol or saline included degeneration and necrosis of few myofibers with associated minimal to slight mononuclear inflammation. Hemorrhages within the fascia with associated inflammation was also present.

**Conclusions:**

PS-341 was considered a tissue irritant when administered by the perivenous and intramuscular routes and no tissue reaction was seen after subcutaneous and intravenous administration.

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**IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:**

By: Lilliam A. Rosario, Ph.D.

**Pharmacology**

PS-341 is a small, dipeptide boronic acid that reversibly inhibits the chymotrypsin-like proteolytic activity of the 20S-proteasome of mammalian cells. The molecule diffuses freely across the cell membrane and binds to the proteasome at significantly lower concentrations than it does to a number of other proteases. The 20S-proteasome binds with several regulatory proteins to create the 26S-proteasome complexes that hydrolyzes proteins that have been marked for destruction by the ubiquitin enzyme cascade. This ubiquitin-proteasome system is responsible for essential elements of homeostatic control within the cell in G<sub>0</sub> and numerous processes through the course of the cell cycle. It is hypothesized that when PS-341 inhibits this system, the cell cycle arrests at the transition G<sub>2</sub>-M, leading inhibited cells to initiate apoptosis. It is also hypothesized this system activates NF- $\kappa$ B by inhibition of proteasome-mediated I $\kappa$ B degradation that, in turn can make cells more sensitive to induction of apoptosis. Thus, some evidence suggests that inhibition of the proteasome can act through multiple mechanisms leading to an arrest of cell growth.

PS-341 inhibited cell growth and in some cases was cytotoxic for human tumor cell types in an *in vitro* cancer screen against a panel of 60 human cancer cell lines. PS-341 also showed activity in both intraperitoneal and subcutaneous implanted hollow fiber/tumor cell models. Further, in both the HT-29 human colon and PC-3 human prostate tumor xenograft model in athymic mice, PS-341 administered IV weekly for 4 weeks (1.0 mg/kg/dose) decreased tumor volume by up to 50% and 65%, for HT-29 and PC-3 xenografts, respectively. The tumor content of PS-341, and relative tissue proteasome activity on these test systems was generally not assessed.

An *ex vivo* proteasome assay was employed as a pharmacodynamic marker of PS-341 presence in the systemic circulation and tissues. When PS-341 is given to animals or to humans, the inhibition of the chymotrypsin-like proteolytic activity can be measured *ex vivo* in the lysate of isolated white blood cells (WBC). It is noteworthy that contamination by red blood cell lysate interferes with this measurement and the assay is difficult to normalize for proteasome content. In the long term toxicity studies in rats and cynomolgus monkeys and in Phase I clinical studies, the mean exposure to PS-341 and inhibition of proteasome activity increased with dose. Activity measured in WBCs from treated animals or humans generally recovers to normal in about 48 hours suggesting elimination of the PS-341 as opposed to *de novo* synthesis of proteasome. Repeat dosing causes significantly greater inhibition compared to a single dose at the same level (about 30% after a single dose compared to almost 99% after seven daily doses in WBCs *ex vivo*). Inhibition could be detected in tissue from colon, muscle, prostate and liver. Inhibition of 20S proteasome activity in the tissues was correlated to the inhibition levels observed in the cellular component of circulating whole blood. However, the inhibition in the liver was significantly greater than in WBCs.

Cells can develop resistance to PS-341 cytotoxicity over time *in vitro*. The mechanism of resistance to PS-341 is not clearly defined, but in one study did not appear to be due to an increase in cellular proteasome content or over-expression of transmembrane molecular pumps. In some cases, the acquired resistance can confer resistance to other proteasome inhibitors.

**Absorption, Distribution, Metabolism, Excretion, and Pharmacokinetics**

The non-clinical pharmacokinetics, absorption, distribution, metabolism, and excretion properties of PS-341 were determined in rats and monkeys after single and multiple dosing. The highest doses used in these studies were in the same range, based on a body surface area adjustment, as doses being evaluated

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in multiple myeloma patients. For the treatment of multiple myeloma, the recommended clinical dose of VELCADE is 1.3 mg/m<sup>2</sup>/dose, administered as a bolus intravenous injection (IV) twice weekly for two weeks (days 1, 4, 8, and 11) followed by a 10-day rest period (days 12-21).

PS-341 permeates easily across cellular membranes in an *in vitro* non-clinical test system. The extent of PS-341 binding to plasma proteins in rat (85%), cynomolgus monkey (72%), and human (83%) was similar across the three species over the concentration range of \_\_\_\_\_ µg/mL. Further, *in vitro*, partition of [<sup>14</sup>C]-PS-341 was higher in plasma than red blood of rat (~40%), cynomolgus monkey (~15%), and human blood (~30%). After IV administration of [<sup>14</sup>C]-PS-341, total radioactivity (consisting of PS-341 and metabolites) in rat and cynomolgus monkey red blood cells was higher than in plasma at all sampling time points. These results suggest that, PS-341 and/or metabolites may associate with the cellular components of blood, and are consistent with the extensive tissue distribution and slow elimination of PS-341 related radioactivity.

were used to quantify [<sup>14</sup>C]-PS-341 and its metabolites during pharmacokinetic (PK) studies. The tissue:plasma concentration ratios in most tissues were greater than one suggesting rapid movement of radioactivity from the vascular compartment into all tissues. The highest concentrations of radioactivity were found in the organs of metabolism and excretion (i.e., liver and kidneys) in rats and monkeys with peak concentrations occurring at 1-3 hours postdose. Radioactivity was detected in the brain of monkeys but not rats. Selected tissues (including heart) and the carcass still contained radioactivity at 144 hours postdose.

PS-341 was extensively metabolized by rats, cynomolgus monkeys, and humans. The major metabolites detected in rat bile (M1, M2, M10, M14, and M15), monkey feces (M1, M2, and M15), and human plasma (M1, M2 and M4) after IV administration of PS-341 were qualitatively similar among the species. These major metabolites were formed by deboronation of PS-341 (mediated by CYP3A4 and 2D6 forming M1 and M2), followed by hydroxylation of the corresponding acid. Thus, PS-341 was primarily metabolized via cytochrome P450 (3A4 and 2D6) and not via Phase II pathways e.g. glucuronidation and sulfation. Representative deboronated PS-341 metabolites formed *in vitro* and *in vivo* have been shown to be inactive as 20S proteasome inhibitors.

PS-341 did not induce the activities of CYP3A4 and 1A2 in primary cultured human hepatocytes. PS-341 was a poor inhibitor of recombinant human CYP P450 isozymes 1A2, 2C9, 2C19, 2D6, and 3A4 with IC<sub>50</sub> values of >18 µM (~7 µg/mL). These IC<sub>50</sub> values are higher than the observed PS-341 C<sub>max</sub> concentration seen in cancer patients (509 ng/mL (range=109-1300 ng/mL)). However, the potential increase or decrease in PS-341 activity by potent inducers or inhibitors of CYP3A4 and 2D6 has not been assessed.

Using carbonyl labeled [<sup>14</sup>C]-PS-341, biliary excretion was established to be the primary route of elimination of radioactivity in rats. In monkeys, both urinary and biliary routes contributed to excretion. Selected tissues (gastrointestinal tract, heart, kidneys, liver, lungs, and pancreas) and the carcass still contained administered radioactivity at 144 hours postdose after single IV administration. The slow elimination and incomplete recovery of administered radioactivity in rats and cynomolgus monkeys are probably due to the extensive tissue distribution and retention of PS-341 and metabolites and slow release of the radioactive material from the tissues. To date, the effect of retained PS-341 and/or metabolites has not been investigated. However, it is noteworthy that PS-341 metabolites do not appear to exert proteasomal inhibition as assessed *in vitro*, so it is unlikely, that metabolites may significantly augment drug activity even if retained.

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Single and multiple dose pharmacokinetic (PK) studies of PS-341 in rats and cynomolgus monkeys were conducted. After single dose IV administration to rats and cynomolgus monkeys, plasma concentrations of PS-341 declined in a biphasic manner with a rapid distribution phase followed by a longer terminal elimination phase. The terminal plasma elimination half-life in cynomolgus monkeys averaged 8 to 10 hours. The area under the plasma concentration-time curve (AUC) increased in a dose-dependent manner over the test range of up to 1.2 mg/m<sup>2</sup> in both species. After multiple doses of PS-341 (twice weekly for 2 weeks followed by 1 week rest), drug exposure with increasing dose was more linear in monkeys compared to rodents; the explanation for this difference is not known. No gender differences were observed in either species. There was a decrease in clearance that resulted in an increase in the terminal elimination half-life (t<sub>1/2</sub>) and AUC (3-4 fold) in rats and cynomolgus monkeys, suggesting drug accumulation. An increase in half-life and AUC and decrease in clearance were also observed in solid tumor patients as below.

Parameter	Dose (mg/m <sup>2</sup> )	Rat (time)	Cynomolgus Monkey (time)	Human (time)
Study #		PK800	PK 888	M34100-27
C <sub>max</sub> (ng/mL)	1.0			157±134 (cycle 1, day 1) 126±87.6 (cycle 1 day 8)
	1.2	13.3 (week 1), 38.1 (week 26) <sup>A</sup>	81±25(week 1), 116±15 (week 38)	
AUC <sub>0-24</sub> (hr*ng/ml)	1.0			30.1±15.3 (cycle 1, day 1), 54.0±14.6 (cycle 1, day 8)
	1.2	65.1 (week 1), 163 (week 26) <sup>A</sup>	51±11 (week 1), 170±33 (week 38)	
Protein binding (%)		85	72	83

<sup>A</sup>High dose was reduced from 1.2 mg/m<sup>2</sup> to 0.9 mg/m<sup>2</sup> on study day 28/29 due to toxicity.

After multiple doses of PS-341 (twice weekly for 2 weeks followed by 1 week rest), pharmacodynamic evaluation, using an *ex vivo* assay, generally showed a dose-dependent decrease in the mean 20S proteasome chymotryptic:tryptic ratio from 30 min to 48 hours following dosing in both rats and monkeys. The maximum decrease in mean ratio activity was observed at 10 and 30 min postdose; partial recovery of mean 20S ratio activities to pre-dose levels was observed by 24-72 hours postdose.

#### Toxicology

Rodents were administered PS-341 as a single dose, weekly x 8, twice weekly for 2 and 26 weeks. The 9-cycle 26-week study is discussed in detail below. PS-341 was administered IV at 0.3, 0.6, and 1.2 mg/m<sup>2</sup> over 5 or 9 three-week cycles (i.e., twice-weekly administrations for 2-weeks followed by a 1-week rest period) to Sprague Dawley rats. On Day 28/29, the high dosage was decreased from 1.2 to 0.9 mg/m<sup>2</sup> due to toxicity. Traditional toxicologic parameters, as well as neuropathological evaluations, toxicokinetic, and proteasome activity were assessed.

PS-341-related mortality was observed at ≥0.9 mg/m<sup>2</sup> (day 50-day 197) and was due primarily to hematopoietic (bone marrow hypocellularity), gastrointestinal (hyperplasia and necrosis), and lymphoid system debilitation (lymphocytic depletion, atrophy, and necrosis of lymph nodes, spleen and thymus). Histopathological changes were observed in the heart (inflammation, hemorrhage, and necrosis), liver (hypertrophy and necrosis), lung (necrosis and inflammation), kidney (necrosis and degeneration), sciatic nerve (necrosis), and spinal cord (inflammation); in general similar findings albeit with less severity were observed in scheduled deaths. Animals dosed ≥0.9mg/m<sup>2</sup> surviving to week 26 (end of treatment), exhibited multifocal neurotoxicity including brain dilatation, and degeneration of dorsal and ventral root ganglia, peripheral nerves, and spinal cord. Chronic progressive nephropathy was generally observed at

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26 weeks at all doses; males appeared to be more susceptible to kidney changes. Histopathological changes in cardiac tissue included increased incidence of perivascular necrosis (at  $\geq 0.6\text{mg/m}^2$ ), myocardial degeneration, hemorrhage, and inflammation. Thrombocytopenia was observed at all PS-341 dose levels. Following the 8-week recovery period, myocardial and vascular inflammation, cardiac necrosis and chronic progressive nephropathy were still observed at all doses; the incidence of findings was not dose-dependent. There appeared to be some indication of reversibility of other findings at this time.

Monkeys were administered PS-341 as a single dose, for 24 hours, daily X 13 days, twice weekly for 2 weeks, and twice weekly for 4- and 13-three week cycles. The 13-cycle study is discussed in detail below. PS-341 was administered IV at 0.6, 0.9, and 1.2  $\text{mg/m}^2$  over 13 three-week cycles (i.e., twice-weekly administrations for 2-weeks followed by a 1-week rest period) to cynomolgus monkeys. PS-341-related mortality was observed at dosages  $\geq 0.9\text{ mg/m}^2$ . The predominant findings in these animals were multifocal neurotoxicity (including brain necrosis and swelling, and degeneration of axons and myelin of dorsal root ganglia, peripheral nerves and spinal cord), severe anemia (bone marrow hypocellularity), thrombocytopenia, cardiotoxicity (necrosis, inflammation, and hemorrhage), and gastrointestinal intolerance (diffuse mucosal hyperplasia) and dehydration; in general similar findings albeit with less severity were observed in scheduled deaths. Neurotoxicity was documented as evidence of lack of neurological reflex observed at multiple sites at all dose levels in animals which survived to 38 weeks (end of dosing period). The incidence of histopathological findings demonstrating neurotoxicity was reduced following 8 weeks of recovery. The incidence of histopathological changes in cardiac tissue, including necrosis and inflammation was minimal and not dose dependent; the severity of cardiac findings was not reported. Kidney findings (hypertrophy/degeneration, glomerulonephropathy, inflammation and the presence of hyaline casts) were observed at  $\geq 0.9\text{ mg/m}^2$  PS-341; males appeared to be more susceptible to kidney changes. Lymphoid atrophy and/or necrosis was exhibited in thymus, spleen, lymph nodes and gut-associated lymphoid tissue. In addition, necrosis and atrophy of the gastrointestinal tract was observed in monkeys surviving to 38 weeks.

Previous studies support the animal models (rat and monkey), dose levels (high dose of  $1.2\text{mg/m}^2$  for both species), and schedules of 9- and 38-three week cycle studies as conducted in rodents and monkeys, respectively. In monkeys and rodents there exists only a small dose margin between lethality at doses  $\geq 0.9\text{mg/m}^2$  and the MTD or HNSTD of  $0.6\text{mg/m}^2$  following administration of PS-341 when dose is adjusted by body surface area. It is noteworthy that the lethal dose and MTD are the same in both species. Cardiac changes were observed in rodents and monkeys; these findings were not dose dependent. In rodents, cardiac findings did not appear to reverse after 8-weeks post drug administration; severity of cardiac findings was not reported. Neurotoxicity exhibited in monkeys and rodents was multifocal and included degeneration of axons and myelin of dorsal root ganglia, peripheral nerves and spinal cord; monkeys appeared to be more susceptible to the neurotoxic effects of PS-341 compared to rodents. Neurotoxicity continued to be exhibited following recovery periods in monkeys and rodents. Clinical observations of tremors and reduced motor activity were exhibited in monkeys; rodents also exhibited reduced motor activity. Both species exhibited hematopoietic, gastrointestinal and lymphoid system debilitation and glomerulonephropathy. Dose- and schedule-dependent changes in AUC and  $C_{\text{max}}$  were also exhibited in both species. However, exposure in high dose rodents was non-linear (approximately  $\frac{1}{2}$  relative increase exposure seen at the mid-dose and low-dose when AUC was normalized with dose). Drug exposure with increasing dose was more linear in monkeys compared to rodents; the explanation for this difference is not known. Toxicokinetic data should be considered general estimates as a result of several factors described in above review. Drug accumulation was observed in both species. Female decedent rats appeared to exhibit a greater degree of toxicity as compared to males. However, pharmacokinetic data were similar between gender; differences in toxicity cannot be attributed to differences in pharmacokinetics. The tissue distribution, steep dose response curve, and the spectrum of toxicities suggest that PS-341 may cause toxicity through mechanisms other

than solely by inhibition of the 20S-proteasome. PS-341 is a substituted dipeptide, and as such, may interact with other cellular sites.

The Sponsor alleged that even though dedicated safety pharmacology studies were not conducted to assess the respiratory and central nervous systems, the data collected in the 6-month rat and 9-month monkey toxicity studies suffices to evaluate the effects of PS-341 in these systems. Toxicology studies included some neurological examinations and specialized histopathology of peripheral nerves, dorsal root ganglia, spinal cord, brain and skeletal muscles, and immunohistochemistry for apoptotic cells in the dorsal root ganglion in both studies. Whereas the histological evaluations of the nervous system may be adequate to document the presence of significant toxicity, it is not accepted that the routine assessment of respiratory function within the toxicology studies is enough to fully investigate and draw conclusion regarding the effects of PS-341 on the respiratory system.

Cardiovascular safety pharmacology studies were conducted in cynomolgus monkeys with follow-up investigative studies conducted *ex vivo* and in mice. In the initial study, telemetered monkeys (1/sex) were administered PS-341 IV at 1.2 mg/m<sup>2</sup> and 32 days later at 3.6 mg/m<sup>2</sup>. In another study in telemetered monkeys (n=1 male/dose), PS-341 was administered IV at doses of 1.2, 2.4, 3.0, and 3.6 mg/m<sup>2</sup>, respectively. PS-341 increased heart rate ( $\geq 1.2$  mg/m<sup>2</sup>) and decreased mean arterial pressure ( $\geq 2.4$  mg/m<sup>2</sup>). At dosages  $\geq 3.0$  mg/m<sup>2</sup> the animals were euthanized moribund by 12-14 hours postdose. Thus, dosages  $\geq 3.0$  mg/m<sup>2</sup> resulted in initial physiologically significant heart rate elevations, which preceded a profound progressive hypotension, bradycardia, and moribundity.

To further investigate the cardiovascular effects of PS-341 in the monkey, a study was conducted in fully instrumented anesthetized cynomolgus monkeys. Animals (1/sex/dose) were administered PS-341 IV at 0.36, 3.60, and 6.00 mg/m<sup>2</sup>, respectively. The Sponsor reported there was no mortality associated with PS-341 administration in this study. However, in previous studies in telemetered monkeys, acute lethality was consistently observed within 12 to 14 hours post drug administration whereas in this study monkeys were sacrificed 6 hours post drug administration, before signs of terminal hypotension and imminent mortality were manifested. Thus, this study is inadequate to address issues of drug-associated mortality observed in the previous studies. Similar to findings in the telemetered monkeys, in the anesthetized animals, heart rate was increased (10-40%) and mean arterial pressure dose-dependently decreased (20-100%) at doses  $\geq 3.6$  mg/m<sup>2</sup>. Additionally, ventricular contractility increased gradually to a peak level of approximately 300% 5 hours after dosing of 3.6 mg/m<sup>2</sup> PS 341. Further, there is a general trend toward increasing (28-69%) cardiac output in monkeys receiving  $\geq 3.6$  mg/m<sup>2</sup>. The Sponsor proposes that increases in contractility suggest a positive inotropic effect. However, plasma concentration of PS-341 at 6 hours were approximately 10% of that seen at 1 hour post-administration suggesting that the pharmacodynamic effects of PS-341 is not directly related to plasma concentration but instead may be dependent upon intracellular or 'target bound' kinetics. Moreover, PS-341 and metabolites have been shown to be sequestered in the myocardium and cardiac necrosis was observed following repeated dosing at 1.2 mg/m<sup>2</sup>. These findings suggest the PS-341-induced cardiac effects may be dependent on or explained by the local disposition of the drug.

Altogether, these data show that PS-341 increased heart rate ( $\geq 1.2$  mg/m<sup>2</sup>), decreased mean arterial pressure ( $\geq 2.4$  mg/m<sup>2</sup>), increased ventricular contractility ( $\geq 3.6$  mg/m<sup>2</sup>), and increased cardiac output ( $\geq 3.6$  mg/m<sup>2</sup>). At dosages  $\geq 3.0$  mg/m<sup>2</sup> PS-341 monkeys in the telemetered studies were euthanized moribund by 12-14 hours postdose. Based on these data, it is not clear what the Sponsor means by "The lack of toxicologically significant findings correlating with those in the telemetry studies described above is best explained by the routine post-operative support provided to the animals which included the maintenance of body temperature by a water jacketed heating device and exposure to a heating lamp during the duration of the experiment". The sequelae of cardiac events are similar in all monkey studies. Furthermore, the difference in reported mortality is easily explained by the inadequate study design in the

anesthetized monkey study, precluding the observation of animals until the time (12-14 hours post drug administration) where signs of progressive terminal hypotension were reported in the telemetered monkey studies. Thus, there are PS-341-induced cardiovascular effects and the effect of body temperature maintenance in PS-341-induced mortality is unknown.

To further investigate the possible mechanisms underlying the initial tachycardia, followed by progressive bradycardia and hypotension observed in telemetry studies, PS-341 was administered IV (0.3, 0.9, 1.0, 9.0, and 30.0 mg/m<sup>2</sup>) to female BALB/c mice (3-5 mice/group). The Sponsor reported in the Overall NonClinical Summary that at 30.0 mg/m<sup>2</sup> there was initial tachycardia followed by a precipitous decrease in heart rate and moribundity within 6 to 8 hours; there was no mention of mortality in the study report. It is notable that the general profile of cardiac changes in the mouse does not appear to mirror those detected in the monkey. In mice, heart rate increases were transient, up to 30 minutes post-dose, followed by prolonged bradycardia from 30 minutes post-dose to sacrifice. In the monkey, heart rate was still increasing by 6 hours (end of monitoring) and progressive hypotension was observed beginning 1-hour post drug administration. Further, lethal doses in mice are 10-fold higher compared to lethal doses in monkeys (30.0 mg/m<sup>2</sup> versus  $\geq 3$  mg/m<sup>2</sup>, respectively) questioning whether the mouse is an appropriate species in which to investigate PS-341-induced lethal cardiovascular effects.

Following administration of 30.0 mg/m<sup>2</sup> PS-341 in mice, there was a decrease in body temperature (5°C). While changes in heart rate were attenuated in mice with the maintenance of body temperature, the effects of body temperature maintenance on PS-341-induced cardiovascular effects in monkeys were not conclusively shown; hyperthermia (1°C) was detected in the monkey. While the Sponsor asserts that hypothermia was the causative factor for changes in cardiac functioning following PS-341 administration, monkey data do not support this perspective.

Further studies in mice investigated the etiology of cardiovascular adverse events through the co-administration of atropine (0.5 mg/kg) and PS-341 (10 mg/kg) to evaluate possible vagal nerve mediated effects. The Sponsor asserted that atropine did not attenuate decreases in heart rate following PS-341 administration. However, graphic representation of the data appears to indicate that atropine may partially attenuate PS-341-induced decreases in heart rate; lack of individual data precluded statistical analysis. These data suggest that increased parasympathetic vagal tone may contribute to the bradycardia observed in mice. The Sponsor only tested 1 dose of atropine, given results showing possible partial attenuation of heart rate changes, additional higher doses of atropine would provide a better assessment of the involvement of the vagus in the PS-341-induced bradycardia.

The Sponsor also conducted an *ex vivo* perfused heart preparation following a single IV bolus of PS-341 (30 mg/m<sup>2</sup>) or saline to mice. The Sponsor concluded that there were "no statistically significant difference in heart rate and force of contraction compared with untreated controls indicating there was no direct effect of PS-341 on the heart". Even though, it is agreed that there are no statistically significant differences in the parameters assayed (left ventricular pressure, end-diastolic pressure, dP/dt<sub>max</sub>, dP/dt<sub>min</sub>, and Tau), there are important trends to be noted. Firstly, based on this experimental design, it is not possible to determine heart rate changes since the pacing rate was set at 7Hz (420 bpm). Thus, the Sponsor's conclusions regarding heart rate are not supported. Second, in PS-341 treated hearts, left ventricular pressure was slightly increased (34%) with a concomitant increase in the force of contractility (dP/dt<sub>max</sub>; 27%). Although not statistically different, these results support a trend of increased contractility after administration of PS-341. Increases in contractility were observed in anesthetized monkeys. Finally, in monkeys, it has been shown that PS-341 and metabolites are sequestered in the myocardium and low concentrations were still measurable 6 days after a single dose of PS-341 (2.4 mg/m<sup>2</sup>). Even though we do not currently have any distribution data on mice, these results suggest that the PS-341-induced cardiac effects may be explained by the local disposition of the drug. Further, histopathological findings of irreversible heart necrosis in monkeys chronically treated with 1.2 mg/m<sup>2</sup>

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PS-341 suggest a direct effect of PS-341 on the heart. In summary, these data suggest that there is a significant potential for adverse cardiovascular events following the administration of PS-341 at doses  $\geq 3.0 \text{ mg/m}^2$ .

These data on the cardiovascular effects of PS-341 indicate that acutely lethal IV dosages of PS-341 are associated with increases in heart rate, decreased mean arterial pressure, and ultimately terminal hypotension. The effect of body temperature maintenance on amelioration of heart rate changes was shown in mice, however, the findings in monkeys were inconclusive. Also, the same PS-341 induced-sequelae of cardiac events are not observed in mice and monkeys. Further, given the disparity in lethal doses in monkeys and mice ( $\geq 3.0 \text{ mg/m}^2$  versus  $30 \text{ mg/m}^2$ , respectively) and the lack of data on drug distribution and exposure in mice, it is unclear whether the mouse is an appropriate species in which to investigate PS-341-induced cardiovascular effects.

In conclusion, given the undefined etiology of the cardiovascular effects seen in multiple non-clinical studies (changes in contractility, mean arterial pressure, and heart rate in acute dosing studies, and cardiac necrosis with chronic dosing), as well as the occurrence of cardiovascular adverse events in the clinic, additional studies appear warranted. Previous studies in monkeys were inconclusive because post-dose observation periods were truncated and not of sufficient duration to encompass the time of maximum cardiovascular effects and pre-terminal changes. Given the narrow safety margin between the recommended clinical dose ( $1.2 \text{ mg/m}^2$ ) and lethality in non-clinical studies ( $3.0 \text{ mg/m}^2$  in monkeys), it is recommended that the sponsor perform additional studies to investigate the factors associated with PS-341 induced lethality at 12 -14 hours post-dose. Since PS-341 promotes dissimilar effects in monkey and mouse, future studies should be conducted in monkeys, the species that appears to most closely model the human response. The Sponsor should perform studies which utilize a broader spectrum of cardiovascular and neuronal agonists and antagonists, and attempt to identify the cardiac cell type(s) that are most effected following PS-341. This would provide further insight into the mechanism by which this drug causes cardiovascular affects, thereby providing a potential clinical intervention in the event of an overdose. Additionally, future studies need to incorporate neuronal assessments to identify or rule out CNS involvement in these phenomena. In the meantime, close monitoring of patients with a history of cardiovascular problems and those presenting with new cardiovascular problems appears warranted to minimize the potential for adverse events.

#### **Genotoxicity**

To assess the genotoxicity and clastogenicity of PS-341, 3 *in vitro* tests (a \_\_\_\_\_ assay and two bacterial reverse mutation assays) and an *in vivo* test ( \_\_\_\_\_ test), were performed. PS-341 was not mutagenic in bacterial reverse mutation assays and the \_\_\_\_\_ assay. Although PS-341 did not induce numerical chromosome aberration in Chinese hamster ovary cells, PS-341 was positive for the induction of structural chromosome aberration, with and without metabolic activation. The Sponsor proposes the positive response in the \_\_\_\_\_ assay may be due to the interruption of the cell cycle and related to the pharmacological action of proteasome inhibition.

#### **Reproductive Toxicology**

The potential reproductive toxicity (Segment II) of PS-341 was evaluated in the Sprague-Dawley rat and New Zealand White rabbit. These studies were conducted with daily IV administration during the period of organogenesis (gestational day 6-17, inclusive in the rat and gestational day 7-19, inclusive in the rabbit) rather than the twice-weekly clinical regimen. Doses for a definitive rodent study were based in a range-finding study in time-mated Sprague-Dawley rats administered PS-341 once daily IV at 0, 0.3, 0.6, 0.9, and  $1.2 \text{ mg/m}^2$ . Results showed maternal lethality demonstrating the steep toxicity curve for PS-341. The only viable dose tested,  $0.3 \text{ mg/m}^2/\text{day}$  was not maternal, embryo, or fetal toxic. Based on these findings, PS-341 was administered (definitive study) once daily IV at dosages of 0, 0.15, 0.30, and 0.45

mg/m<sup>2</sup>. No lethality or significant clinical signs were observed. The only indications of maternal toxicity were decreased body weight and food consumption in the early stages of drug administration at 0.45 mg/m<sup>2</sup>. Examination of the fetuses exposed to PS-341 during organogenesis showed no significant effects of the drug on skeletal and visceral development. No embryo-fetal toxicity or lethality was seen at any dose, even when indications of maternal toxicity were present. The NOAEL for maternal and fetal effects was 0.3 mg/m<sup>2</sup>/day and this daily IV dosage is approximately 0.2 times the proposed twice-weekly IV clinical dosage of 1.3 mg/m<sup>2</sup>, based on body surface area.

In rabbits, the doses for the definitive developmental toxicity (0, 0.11, 0.3, 0.6 mg/m<sup>2</sup>) were based on no clear evidence of embryoletality or fetotoxicity at doses up to 0.6 mg/m<sup>2</sup>/day in a dose ranging study. An additional study was conducted in non-pregnant New Zealand white rabbits where mortality was observed at doses greater than 0.9 mg/m<sup>2</sup>/day PS-341. PS-341-related maternal mortality, abortion, clinical signs and decreased body weight gain and food consumption were observed at 0.6 mg/m<sup>2</sup>. Embryo-lethality was evidenced at 0.6 mg/m<sup>2</sup> by increased numbers of resorptions and a decreased live litter size and lower fetal weights indicating mild fetotoxicity. The incidence of litters and fetuses with major malformations was unaffected by treatment. There was no clear teratologically significant effect upon the incidences of minor external, visceral and skeletal anomalies, or percentage of fetuses with rib and sternbral variants when compared to controls. The NOAEL for maternal toxicity and embryo-fetal development was 0.3 mg/m<sup>2</sup>/day, which is 0.21 times the proposed twice-weekly IV clinical dosage of 1.3 mg/m<sup>2</sup>, based on body surface area. While maternal toxicity is certainly evident at this dose group, the toxicities are mostly minor, such as decreased food consumption and small decreases in weight gain. Clinically, these are not toxicities that would lead to drug cessation. That embryo-fetal lethality and toxicity were noted at doses with minimal maternal toxicity has important clinical ramifications. In humans, treatment with PS-341 could impact the ability to maintain viable pregnancies. It is important that female patients are aware of this potential and will take precautions to avoid pregnancy.

Teratological effects of PS-341 were examined in both the rat and rabbit. In neither species were there significant increases in major skeletal or visceral malformations due to in utero exposure to PS-341 (at doses up to 0.6 mg/m<sup>2</sup>/day. Embryo-fetal toxicity and lethality were seen in the rabbit at 0.6 mg/m<sup>2</sup>/day (half of the proposed clinical dose of 1.3 mg/m<sup>2</sup>). The difference in schedules makes comparison between the two difficult, as the rabbit was dosed daily for 13 days, as opposed to the intermittent schedule used clinically. No formal evaluation of effects on fertility or peri- and postnatal development (Segments I and III, respectively) were conducted. However, based on embryoletality findings in rats and rabbit, as well as the effects of PS-341 on primary and secondary sex organs as observed in the 6-month rat study and the 9-month monkey toxicity study, PS-341 is likely to have a potential negative or adverse effect on fertility. In the 6-month rat toxicity study testicular seminiferous tubule degeneration was observed in males at the highest dosage (0.9/1.2 mg/m<sup>2</sup>), and ovarian luteal cell necrosis was observed in females at all dosages ( $\geq 0.3$  mg/m<sup>2</sup>).

#### ***Special Toxicology Study: Tissue Irritation***

A tissue irritation study was conducted in male New Zealand white rabbits after administration of 1.2 mg/m<sup>2</sup> PS-341 as a single intravenous, perivenous, intramuscular, or subcutaneous injection. PS-341 treatment resulted in a slight increase in incidence of erythema and/or edema following intravenous and intramuscular administration as compared to saline with recovery by 72 hrs post injection. Microscopic changes were observed following perivenous (6/6 rabbits) and intramuscular (1/3) injection. Minimal to moderate perivascular inflammation and edema were observed. At 24 hours post-injection, the PS-341 injection sites of 3/3 animals demonstrated necrosis of isolated keratinocytes in the stratum spinosum. At 72 hours post-injection, 2/3 animals demonstrated minimal epithelial parakeratosis. After intramuscular administration, locally extensive moderate degeneration and necrosis of myofibers were present. Myofibers were swollen, eosinophilic and fragmented with a moderate heterophilic (acute) inflammation. No difference in erythema or edema was noted between mannitol and saline, following perivenous,

intravenous, or subcutaneous administration. Changes after intramuscular administration of mannitol or saline included degeneration and necrosis of few myofibers with associated minimal to slight mononuclear inflammation. Hemorrhages within the fascia with associated inflammation was also present. PS-341 was considered a tissue irritant when administered by the perivenous and intramuscular routes and no tissue reaction was seen after subcutaneous and intravenous administration.

**Route-Specific Toxicology Concerns**

A study utilizing intra-prostatic administration of PS341 was conducted in dogs. Ataxia was observed following a single intraprostatic injection of 0.6 mg/kg (12 mg/m<sup>2</sup>). Single doses of 0.6 mg/m<sup>2</sup> and repeat doses of 0.13 mg/kg (2.6 mg/m<sup>2</sup>) resulted in red stool, diarrhea, emesis, anorexia, and hypoactivity. Mortality (day 2 and 8), ataxia, quadriparesis, rectal bleeding, lesions in the ventral roots of the spinal cord, ileocolic intussusception and necrosis of the termina ileum were observed following administration of 0.13 mg/kg PS 341 bi-weekly for 3 doses.

In this study, mild to severe progressive clinical signs consistent with peripheral neuropathy were observed. Most notable is the apparent retrograde degeneration from the neural plexus of the prostate to the ventral roots of the spinal cord (Wallerian degeneration). This effect is of concern particularly given the local route of administration. The same type of lesion has been observed in both the 4-week and 38-week cynomolgus monkey toxicity studies. In the monkey studies, the lesion was confined to axons associated with the PNS, affected only anatomic pathways carrying sensory nerves, and was not associated with degeneration of the nerve cell bodies of the sensory nerve axons. While in the monkey studies full reversibility was not demonstrated, following an 8- week off-dose period animals appeared to show partial recovery and the lack of cell death in the dorsal root ganglia suggest an eventual recovery.

Intraprostatic administration of PS- 341 resulted in severe neurological dysfunction following single-(0.6 mg/kg; 12 mg/m<sup>2</sup>) or repeat-dose (0.13 mg/kg or 2.6 mg/m<sup>2</sup>; bi-weekly for 3 doses) administration. The Sponsor proposes that "a possible role of the particular mode of administration and/or predisposition of the species of dog to neurological pathology such as coonhound paralysis or acute idiopathic polyradiculoneuritis" was a possible cause. However, this condition is known to be a rare and most frequently associated with a bite from a raccoon. Further, the clinical course and histopathology described appears somewhat different from coonhound paralysis, which usually has significant lymphocytic inflammation. Thus, it is unlikely that the ataxia seen in this dog study is related to this condition. Additionally, the time course strongly suggests an association with PS-341. Additionally the Sponsor asserts that there was no clinical local toxicity in any of the dogs in areas adjacent to the injection site. However, hemorrhage, edema, and granulation was detected in 8 instances in the urinary bladder after intraprostatic administration of PS-341.

**General Toxicology Issues:**

Safety Margin compared to recommended clinical dose:

In monkeys and rodents there exists only a small dose margin between lethality (at doses  $\geq 0.9$ mg/m<sup>2</sup>) and the maximum tolerated dose or HNSTD (0.6mg/m<sup>2</sup> PS-341). It is noteworthy that the lethal dose and maximum tolerated dose are the same in both species. Furthermore, there is no safety margin compared to the proposed clinical dose (1.3 mg/m<sup>2</sup>).

Species	Study <sup>A</sup>	Lethal dose (mg/m <sup>2</sup> )	MTD (mg/m <sup>2</sup> )	Safety Margin
Rat	9-cycles (6-months)	$\geq 0.9$	0.6	<1
Monkey	13-cycles (9-months)	$\geq 0.9$	0.6	<1
Human			1.3 <sup>AB</sup>	

<sup>A</sup> Twice weekly schedule

<sup>B</sup> Recommended dose

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#### Cardiotoxicity:

Cardiovascular safety pharmacology studies conducted in cynomolgus monkeys showed that administration of dosages  $\geq 3.0 \text{ mg/m}^2$  PS-341 (twice the recommended clinical dose) resulted in initial physiologically significant heart rate elevations, which preceded a profound progressive hypotension, bradycardia, and death 12-14 hours postdose. Increased heart rate and decreased mean arterial pressure were also observed at lower doses of PS-341 ( $\geq 1.2 \text{ mg/m}^2$  and  $\geq 2.4 \text{ mg/m}^2$ , respectively).

It appears that changes in cardiovascular parameters reflect pharmacodynamic effects of PS-341 that are not directly related to plasma concentration, but instead may be dependent upon intracellular or 'target bound' kinetics. Moreover, PS-341 has been shown to be sequestered in the myocardium. Cardiac necrosis was observed following repeated dosing at  $1.2 \text{ mg/m}^2$ , further suggesting that PS-341-induced cardiac effects may be dependent on or explained by the local disposition of the drug.

These data on the cardiovascular effects of PS-341 indicate that acutely lethal IV dosages of PS-341 are associated with increases in heart rate, decreased mean arterial pressure, and ultimately terminal hypotension.

#### Neuropathy

Monkeys and rats were dosed for 13-three weeks with a similar schedule as recommended for patients (twice weekly dosing for 2 weeks followed by 1-week rest). Neurotoxicity was multifocal and included brain necrosis, and swelling and degeneration of axons and myelin of dorsal root ganglia, peripheral nerves, and spinal cord. Multifocal nerve degeneration of dorsal root ganglia, peripheral nerves and spinal cord was observed at  $\geq 0.6 \text{ mg/m}^2$  (one half the recommended clinical dose of  $1.3 \text{ mg/m}^2$ ).

#### Cytochrome P450 Regulation

PS-341 prevented the proteasome-dependent degradation of cytochrome P450 2E1 after ethanol induction. This degradation returns intracellular expression of P450 2E1 to constitutive concentrations after induction. Other cytochromes P450 may also be degraded by proteasomes. Thus, PS-341 has the potential to modify the metabolism of a broad range of chemicals by increasing the intracellular concentration of cytochrome P450. This could possibly result in decreased exposure for drugs that are metabolized by cytochrome P450 with a concomitant decrease in efficacy or enhanced conversion of drug to activated forms.

#### Intraprostatic Injection of PS-341:

Intraprostatic administration of PS-341 resulted in severe neurological dysfunction following single ( $12 \text{ mg/m}^2$ ) or repeat-dose ( $2.6 \text{ mg/m}^2$ ; bi-weekly for 3 doses) administration. Most notable is the apparent retrograde degeneration from the neural plexus of the prostate to the ventral roots of the spinal cord (Wallerian degeneration) after local intra-prostatic administration of PS-341. The same type of lesion has been observed in 4-week and 38-week cynomolgus monkey toxicity studies (IV dosing). These data suggest that extravascular extravasation or, local tissue injection of PS-341 in highly innervated tissues, may be associated with particularly severe and progressive neuronal injury.

#### Prion Proteins

Limited evidence suggests the possibility that inhibition of the proteasome could increase the concentration of prion proteins (PrP) in the cytosol of neurons. Normal PrP ( $\text{PrP}^c$ ) undergoes post-translational modifications including folding into a mostly  $\alpha$ -helical secondary structure. As much as 10% of  $\text{PrP}^c$  is processed incorrectly, resulting in its diversion to the ubiquitin-proteasome-system. One malformation of PrP is the formation of  $\text{PrP}^{sc}$ , a misfolded protein that has a predominantly  $\beta$ -sheet secondary structure. It has been postulated that  $\text{PrP}^{sc}$  is the transmissible agent or the predominant part of that agent in a fatal neuro-degenerative disease in sheep and goats that manifests as a spongiform

encephalopathy; analogs in other species include cattle (mad cow disease or bovine spongiform encephalopathy) and humans (Creutzfeldt-Jakob disease). This protein may act as a chaperone protein that is able to refold PrP<sup>c</sup> to PrP<sup>sc</sup>, thus propagating itself and the disease.

It has been shown that treatment of cells transfected with a normal PrP gene with other proteasome inhibitors, such as lactacystin or epoxomicin, results in the accumulation of proteins in the cytosol (Ma and Lindquist, 2002). The concentrations of these proteins, except for PrP<sup>c</sup>, return to normal after the inhibition has been removed. Misfolding of the normal PrP protein occurred with the formation of proteins with a PrP<sup>sc</sup>-like conformation. One possible implication suggest that inhibition of the proteasome pathway could lead to the accumulation of PrP protein followed by the formation of PrP<sup>sc</sup> - like  $\beta$ -sheet proteins that could refold yet more PrP proteins. It is conceivable that this could exacerbate spongiform encephalopathies or even initiate them by inducing the formation of PrP<sup>sc</sup> proteins. PS-341 is found in relatively low concentrations in the brains of animals given a single radiolabeled dose. Nonetheless, after chronic administration, brain necrosis was observed suggesting that PS-341 crosses the blood brain barrier. In talks with the Agency, the Sponsor offered to repeat the above-mentioned studies using PS-341 as the inhibitor, however, this information was not provided with the current NDA.

#### Microarray Data

In the review of supporting materials for this application, it was noted to the Applicant that they had not included in their package a reference from the "open literature" that used complementary whole genome technologies in yeast to investigate their proposed mechanisms of action. In their response, the applicant stated that this publication was not included in the current submission because they were not sure as to the relevance of the findings in yeast cells to the higher eukaryotic cells. Moreover, they added that they have initiated a 'patient-voluntary' pharmacogenomics research component of the Phase 2 clinical study to explore the use of gene chip technology to identify potential candidate genes possibly associated with clinical response. Also, they are expanding the initial exploratory evaluation to additional clinical samples in the ongoing Phase 3 clinical trial. Separate clinical pharmacogenomics efforts are being undertaken by additional institutions and non-clinical work has been initiated in mammalian cell lines that includes both genomic and proteomic analysis of cells treated with their compound.

As a justification for not reporting this information, the Applicant noted that Dr. Steve Galson proposed a 'safe harbor' process for evaluation of pharmacogenomic data at an FDA/Industry forum on Pharmacogenetics and Pharmacogenomics in May of last year. We have requested clarification from Dr. Woodcock on whether the applicant is required to submit this data, albeit not to base a regulatory action. Since it has been proposed that the "safe harbor" mechanism will "help the Agency and industry learn from these data" (Dr. Galson, Pink Sheet March 17, 2003), we would also like to know whether we can request the Applicant to submit the data. An action on this NDA is expected in the near future, we do not expect any regulatory impact based on such data.

#### **Recommendations:**

1. Additional non-clinical studies appear warranted given the undefined etiology of the cardiovascular effects seen in multiple animal studies, as well as the occurrence of cardiovascular adverse events in patients.

Given the narrow safety margin between the recommended clinical dose (1.2 mg/m<sup>2</sup>) and 100 % lethality in non-clinical studies (3.0 mg/m<sup>2</sup> in monkeys), we recommend the sponsor determines the factors associated with PS-341 induced lethality at 12-14 hours post-dose.

Since PS-341 promotes dissimilar effects in monkey and mouse, future studies should be conducted in monkeys, the species that appears to most closely model the human response.

The Sponsor should identify the cardiac cell type(s) that are most effected following PS-341 administration to provide potential clinical interventions in the event of an overdose.

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Future non-clinical studies need to incorporate neuronal assessments to identify or rule out CNS involvement in these phenomena.

2. The Sponsor should conduct a study in cells transfected with a normal PrP gene to determine if administration of PS-341 results in the accumulation of proteins in the cytosol, similar to treatment with other proteasome inhibitors such as lactacystin or epoxomicin, as reported by Ma and Lindquist, 2002. Further, determine if misfolding of the normal PrP protein occurred with the formation of proteins with a PrP<sup>sc</sup>-like conformation. The implications of these findings to the possible initiation and/or exacerbation of spongiform encephalopathies should be addressed.

**Labeling with basis for findings:**

Appendix B

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# Appendix A



## X. APPENDIX/ATTACHMENTS:

**Memorandum** DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH  
DIVISION OF CARDIO-RENAL DRUG PRODUCTS

**FROM:** Anthony G. Proakis, Ph.D., Pharmacologist Reviewer, DCRDP, HFD-110

**THROUGH:** Charles A. Resnick, Ph.D., Pharmacology Team Leader, DCRDP, HFD-110  
Douglas C. Throckmorton, M.D., Director, DCRDP, HFD-110

**TO:** Richard Pazdur, M.D., Director, Div. Oncology Drug Products, HFD-150  
Sean Bradley, Project Manager, Div Oncology Drug Products, HFD-150  
Sandi Leigh Verbois, Ph.D. Div. Oncology Drug Products, HFD-150

**SUBJECT:** Velcade Inj. (PS-341, Millennium Pharmaceuticals); NDA # 21,602

**DATE RECEIVED:** 2/20/03

**DATE COMPLETED:** 4/01/03

## INTRODUCTION

Millennium Pharmaceuticals submitted to the Division of Oncology Drug Products a New Drug Application (NDA # 21,602) for Velcade (bortezomib) for Injection for the treatment of relapsed/refractory multiple myeloma

The Division of Oncology Drug Products is requesting that we evaluate the results of non-clinical pharmacology studies, conducted in cynomolgus monkeys, that showed increases in myocardial contractility at doses that are clinically relevant.

Three study reports were submitted that describe the effects of PS-341 on cardiovascular function in cynomolgus monkeys.

## STUDY DESCRIPTIONS AND RESULTS

### *PS-341: Cardiovascular Effects after Intravenous Administration in Telemetered Cynomolgus Monkeys*

This study, conducted for Millennium Pharmaceuticals by \_\_\_\_\_, assessed the effects of single intravenous doses of PS-341 in cynomolgus monkeys. One male and one female monkey each received an intravenous dose of 0.2 mg PS-341/kg on Day 1 of the study and a second intravenous dose of 0.3 mg PS-341/kg on Day 32 of the study. The animals were monitored for clinical signs of toxicity at periodic intervals following each dose. Electrocardiographic (Lead II) and blood pressure measurements were recorded telemetrically before each dose and continuously for up to 24 hours after the 0.2 mg/kg dose and for 12 hours after the 0.3 mg/kg dose. Approximately 12 hours after the second dose, the animals were sacrificed and necropsied.

The administration of the 0.2 mg/kg IV dose resulted in vomiting by the female monkey approximated 6 hours after dosing on Day 1; the 0.3 mg/kg dose resulted in vomiting approximately 6 hours after dosing in the male monkey and on six occasions (approximately 4.5 to 11 hours postdose) for the female monkey.

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Heart rates and mean blood pressures (results presented as continuous recordings) fluctuated during the predose and post dose periods. A sustained fall in mean blood pressure (~ 20 mmHg) accompanied by a rise in heart rate (~ 40-50 bpm) occurred in the female monkey approximately 6 hours after the 0.2 mg/kg dose. The cardiovascular responses in the female monkey appeared to coincide with the emetic episode in this animal. It is not discernable if the cardiovascular responses were direct effects of the drug or were physiological consequences of the emetic response. A similar delayed blood pressure fall and heart rate increase was seen in this animal following the 0.3 mg/kg dose. Heart rate and blood pressure in the male did not seem to be remarkably changed from predose values following either dose of PS-341.

#### *Cardiotoxicity of PS-341 (NSC-D681239) in the Monkey*

This study was conducted for the National Cancer Institute, NIH, by \_\_\_\_\_ to evaluate the potential cardiotoxicity of intravenous doses of PS-341 in male cynomolgus monkeys. Four monkeys were administered a single IV dose of PS-341 (0.1, 0.2, 0.25 or 0.3 mg/kg) and the animals were observed for clinical signs of toxicity up to 12 hours postdose and then twice daily for up to 8 days postdose. Heart rate, blood pressures, body temperature and ECGs were recorded from all animals via implanted radiotelemetry devices.

The 0.1 mg/kg dose elicited no adverse effects. The monkey given the 0.2 mg/kg dose vomited approximately 6 hours after dosing. After the 0.25 mg/kg dose, the animal vomited approximately 4.5 and 5.5 hours postdose. The fourth animal, which received the 0.3 mg/kg dose, became lethargic experienced neuromuscular tremors, developed diarrhea, laid down in its cage and became unresponsive. The latter two animals given the 0.25 and 0.3 mg/kg doses were euthanized approximately 13-14 hours following dosing.

Heart rates increased in all 4 animals following administration of PS-341. The mean blood pressure in the animal given the 0.1 mg/kg dose showed little to no change from predose levels; however, a fall in mean blood pressure was observed after administration of 0.2, 0.25 and 0.3 mg/kg of PS-341. Blood pressure returned to normal levels after the 0.2 mg/kg dose but did not follow diurnal patterns for approximately 4 to 5 days after dosing. The elevated heart rate seen with 0.1 and 0.2 mg/kg returned to baseline after 2 to 4 days post dose. The animal receiving the highest dose became extremely hypotensive and remained so until euthanized. No effect on the electrocardiogram was seen following any dose of PS-341.

It appears that the increased heart rate following PS-341 administration is a compensatory response to the drug-induced hypotension.

#### *A Study to Determine the Effects of PS-341 on Cardiovascular Function after Intravenous Administration to Anesthetized Cynomolgus Monkeys*

This study was conducted for Millennium Pharmaceuticals by \_\_\_\_\_ to evaluate the effects of intravenous PS-341 on cardiovascular function in anesthetized cynomolgus monkeys. Three male and three female cynomolgus monkeys were anesthetized with isoflurane and instrumented to record heart rate, arterial blood pressure, pulmonary arterial blood pressure, central venous pressure, left ventricular pressure and contractility (LVdp/dt), cardiac output, body temperature and electrocardiogram. Single PS-341 doses of 0.03, 0.3 and 0.5 mg/kg were administered intravenously to 1M and 1F per dose. The animals were monitored for 6 hours after dosing. Venous blood samples were obtained at baseline and one and six hours post dose for measurement of plasma concentrations of PS-341.

No animals died during the 6-hour postdose observation period. The electrocardiogram was unaffected by PS-341 treatment. At the 0.03 mg/kg dose, heart rates fluctuated  $\pm 10\%$  from mean baseline values over the 6-hour period. This dose induced a gradual increase (10-25%) in blood pressure that peaked at 3 to 4 hours following dosing. At the 0.3 mg/kg dose, both animals experienced an initial decrease (10-20%) in arterial pressure during the first hour after dosing with blood pressure continuing to decline over

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the 6 hour observation period. Heart rate in the male at the 0.3 mg/kg dose increased gradually and at 5 hours post dose was about 50% higher than baseline value. Heart rate in the female treated with 0.3 mg/kg of PS-341 increased modestly (~10%). In both animals given the 0.5 mg/kg dose, a biphasic blood pressure response was observed, an initial increase (30-50%) above baseline value during the first 2 hours post dose followed by a decrease in blood pressure from baseline. Heart rate in the male monkey showed a gradual decrease (~10%) over the 6 hours period whereas a gradual increase (up to 40% from baseline) was seen in the female given the 0.5 mg/kg dose.

Maximal LVdp/dt increased by 20-50% above baseline in both animals given the 0.03 mg/kg dose and increased up to 300% above baseline in both males and females after the 0.3 mg/kg and 0.5 mg/kg doses.

Cardiac output remained relatively unchanged in each animal after the 0.03 mg/kg dose but increased above baseline values after the 0.3 and 0.5 mg/kg doses.

### SUMMARY AND EVALUATION

In the two studies conducted in conscious cynomolgus monkeys, a steep dose-response for toxicity was observed for PS-341. No adverse effects were observed after an IV dose of 0.1 mg PS-341/kg. Doses  $\geq$  0.2 mg/kg IV caused emesis and a dose of 0.3 mg/kg IV produced neuromuscular tremors, diarrhea and unresponsiveness that necessitated early sacrifice of the animals.

In conscious monkeys, IV doses  $\geq$  0.2 mg PS-341/kg caused a drop in mean arterial blood pressure and increases in heart rates from baseline levels. The increases in heart rate generally coincided with the blood pressure fall and appears to reflect a compensatory response to the drug-induced hypotension.

In anesthetized monkeys, doses up to 0.5 mg/kg of PS-341 (which were emetic in conscious animals) were explored for effects on cardiovascular function without causing vomiting. The lowest dose (0.03 mg/kg IV) produced minor fluctuations in mean blood pressures and heart rates. A reduction in mean blood pressure from baseline level occurred in the male and female monkeys treated with the 0.3 mg/kg IV dose and was accompanied by increases (50% in the male and 10% in the female) in heart rates from baseline. Myocardial contractility (LV dp/dt) in anesthetized monkeys (not measured in conscious animals) increased above baseline after the 0.3 and 0.5 mg/kg doses of PS-341.

A consistent finding among these 3 studies is that PS-341 causes a fall in mean blood pressure following IV doses  $\geq$  0.2 mg/kg. The increases in heart rate and myocardial contractility appear to coincide temporally with the induced hypotension and most likely reflect compensatory cardiovascular responses. However, a direct (positive inotropic) effect of PS-341 on the myocardium cannot be totally excluded by these experiments alone. Typically, in vitro isolated heart or isolated myocardial preparations are used to determine direct inotropic (positive or negative) effects of drugs.

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**STATISTICAL REVIEW**

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# Appendix B

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