

Other studies

(I) Toxicokinetic Study Report Supporting a 26-Week Intravenous Injection Toxicity Study of PS-341 in the Sprague- Dawley Rat (Final Data). Module 4, rpt-00144.

This report is in support of toxicity study CTBR 57285 conducted in male and female Sprague-Dawley rats.

Key study findings: The exposures to PS-341 in week 14 were 2-5 fold higher than those observed in week 1 indicating possible accumulation of the drug.

Methods: The animals were dosed twice weekly at 0.0, 0.05, 0.10 or 0.2 mg/kg for two weeks followed by 1 week rest period. This three weeks treatment cycle continued for 26 weeks. Plasma samples were taken at few time points (0.5, 1, 6, and 24 hours postdose) from 3 animals/sex/group.

Results:

Toxicokinetic Exposure Parameters for Weeks 1, 14, and 26 are shown below.

Dose (mg/kg)	Week	Observed Cmax (ng/mL)	AUC ₀₋₂₄ (ng.hr/mL)	AUC ₀₋₂₄ /dose (ng.h/ml/mg.kg)
0.05	1	---	14.5	290
0.10	1	---	27.4	274
0.20	1	---	65.3	327
0.05	14	---	84.6	1690
0.1	14	---	145	1450
0.2*	14	---	137	913
0.05	26	---	84.7	1690
0.1	26	---	134	1340
0.2*	26	---	163	1087

* High dose was reduced from 0.2 mg/kg to 0.15 mg/kg (from 1.2 mg/m² to 0.9 mg/m²) on study day 28/29 due to toxicity.

The exposure to PS-341 increased in a dose dependent manner in week 1. The exposures observed in week 14 were approximately 2-5 fold higher than that observed in week 1, indicating possible accumulation of the drug. The exposures observed in week 26 were similar to those observed in week 14 indicating that steady state levels of systemic exposure may have reached. During weeks 14 and 21, C_{max} was approximately linear with dose. However, AUC at the highest dose (0.2/0.15 mg/kg) was reduced compared to the low and mid dose when normalized. The reason for the non-linearity in AUC at the high dose for week 14 and 26 is unknown.

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Final Report Amendment No. 1

The purpose of the amendment was to correct errors in the transfer of numbers from data sheets. These changes are noted in the table below (copied from the sponsor's submission dated April 1, 2003) but do not affect our conclusions.

Note: Non GLP but QA statement is included in the Amendment report.

Toxicokinetic Exposure Parameters for Weeks 1, 14, and 26

Dose (mg/kg)	Week	Observed C _{max} (ng/mL)	Estimated ^a C _{max} (ng/mL)	AUC ₀₋₂₄ (ng*hr/mL)	AUC ₀₋₂₄ /dose (ng*hr/mL/mg*kg)
0.05	1	—	1.84	14.5	290
0.10	1	—	5.52	27.4	274
0.20	1	—	13.3	65.3	327
0.05	14	—	7.14	84.6	1690
0.10	14	—	15.1	145	1450
0.20	14	—	30.5	137	685
0.05	26	—	5.80	84.7	1690
0.10	26	—	11.5	134	1340
0.20	26	—	38.1	163	817

a. Estimated for time = 0.

In the above table, AUC₀₋₂₄/dose calculations at high dose for weeks 14 and 26 are incorrect. The high dose was reduced from 0.2 mg/kg to 0.15 mg/kg on study day 28/29 due to toxicity (see toxicology study CTBR 57285). Sponsor had not mentioned this in this toxicokinetic study and has done the calculation based on dosing at 0.2 mg/kg/day. The correct calculations are shown below.

Toxicokinetic Exposure Parameters for Weeks 1, 14, and 26

Dose (mg/kg)	Week	Observed C _{max} (ng/mL)	AUC ₀₋₂₄ (ng.hr/mL)	AUC ₀₋₂₄ /dose (ng.h/ml/mg.kg)
0.20	1	—	65.3	327
0.15	14	—	137	913
0.15	26	—	163	1087

(2) Toxicokinetic Study Report Supporting a 38-Week (13 Cycles) Intravenous Injection Toxicity Study of PS-341 in the Cynomolgus Monkey. Module 4, rpt-00039.

This report is in support of toxicity study CTBR 57284 in male and female cynomolgus monkeys.

Key study findings: A dose-dependent increase in C_{max} and AUC were observed. There was an accumulation of drug in the plasma with repeated doses. There was no accumulation of effect on 20S activity over repeated doses.

Methods: PS- 341 was administered by intravenous (IV) injection twice weekly at 0.05, 0.075 or 0.1 mg/kg for two weeks followed by a 10- day rest period (1 cycle), for 38 weeks (13 cycles) as shown below.

Schedule of Dosing Cycles and Toxicokinetic and Pharmacodynamic Sample Collection Days

Cycle	Week	Dose	
1	1	1 ^a	2
	2	3	4
	3	off	off
2	4	1	2
	5	3	4 ^a
	6	off	off
n			
		off	off
13	37	1 ^a	2
	38	3 ^a	4
	39	off	off

a. Samples taken for TK/PD analysis.

A subset of 3 monkeys/sex/dose in the 0.05 and 0.075 mg/kg group, and 6 animals/sex in the 0.1 mg/kg dose group were sampled for evaluation of the kinetic disposition of PS-341 in plasma, at predose, 0.17, 0.5, 1, 6, and 24 hours postdose (few time points) in weeks 1, (dose 1, cycle 1), 5 (dose 4, cycle 2), 37 (dose 1, cycle 13), and 38 (dose 3, cycle 13).

Results:

The male and female data are combined since no gender related differences in the disposition of PS-341 were observed.

Summary Toxicokinetic Exposure Parameters for Weeks 1, 5, 37, and 38.

Dose (mg/kg)	Week	Cycle/dose	Estimated			
			C _{max} (ng/mL)	AUC ₀₋₂₄ (hr*ng/mL)	AUC ₀₋₂₄ /dose (hr*ng/mL/mg*kg)	
0.05	1	1/1	Mean	25.4	12.3	246
			SD	10.9	2.69	53.8
	5	2/4	Mean	45.3	45.1	902
			SD	7.9	7.73	155
	37	13/1	Mean	49.4	66.9	1340
			SD	9.18	28.9	577
	38	13/3	Mean	48.2	83.1	1660
			SD	12.4	38.1	761
0.075	1	1/1	Mean	40.5	34.6	462
			SD	9.54	10.4	139
	5	2/4	Mean	82.3	82.9	1110
			SD	16.6	15.2	203
	37	13/1	Mean	56.7	108	1450
			SD	18.7	17	226
	38	13/3	Mean	73.5	140	1870
			SD	13.6	29.5	390
0.1	1	1/1	Mean	80.6	51.3	513
			SD	25.1	10.6	106
	5	2/4	Mean	138	111	1110
			SD	51.4	29.5	296
	37	13/1	Mean	81.7	138	1380
			SD	12.4	46.7	466
	38	13/3	Mean	116	170	1700
			SD	14.5	31.1	331

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A dose-dependent increase in C_{max} and AUC were observed. The mean C_{max} and AUC values increased over time with repeated dosing from week 1 to week 38 indicating an accumulation of the drug in the plasma. The dose normalized C_{max} and AUC were linear for a particular time point. This contrasts to the rat, where the AUC in high dose animals was less than linear. The reasons for this are unknown, but it should be noted that the principal metabolic enzymes (2D6 and 3A4) are not induced by PS-341 in human liver microsomes.

Mean Maximum Percent Inhibition of 20S Ratio Activity in Monkeys

Dose (Week)	% Inhibition (ChT:T)		
	0.05 mg/kg	0.075 mg/kg	0.10 mg/kg
1	60	72	77
5	70	77	80
37	60	71	78
38	54	70	70

ChT:T = ratio of chymotryptic to tryptic activity.

There was no accumulation of pharmacodynamic effect on the 20S proteasome activity after repeated dosing.

Summary of toxicokinetic exposure parameters for weeks 1, 5, 37 and 38.

Dose (mg/kg)	week	Cycle /dose	AUC ₀₋₂₄ (hr*ng/mL)		AUC ₀₋₂₄ /dose (hr*ng/mL/mg*kg)		t _{1/2} (hr)	AUC _{INF} (hr*ng/mL)		AUC _{INF} /dose (hr*ng/mL/mg*kg)	
			Mean	SD	Mean	SD		Mean	SD	Mean	SD
0.05	1	1/1	12.3	2.7	246	54	2.7±0.2	14.5	2.7	290	54
	5	2/4	45.1	7.7	902	155	12.9±2.9	62.3	15.5	1250	309
	37	13/1	66.9	28.9	1340	577	47.9±43.9	116	65	2310	1290
	38	13/3	83.1	38.1	1660	761	55±30.8	156	104	3120	2080
0.075	1	1/1	34.6	10.4	462	139	9.9±3.9	47	17	626	226
	5	2/4	82.9	15.2	1110	203	12.4±3.6	117	25.4	1560	338
	37	13/1	108	17	1450	226	130±77.2	278	133	3710	1780
	38	13/3	140	29.5	1870	390	46.7±12	196	30.1	2620	402
0.1	1	1/1	51.3	10.6	513	106	7.8±3.2	61.3	14	613	140
	5	2/4	111	29.5	1110	296	9.7±2.6	140	45.2	1400	452
	37	13/1	138	46.7	1380	466	95.3±28.4	285	117	2850	1170
	38	13/3	170	33.1	1700	331	53.4±11.7	248	55	2480	550

There was a lot of variation in half-life. Within a given dose group, AUC increased with increase in cycles of dosing indicating an accumulation of the drug.

Final Report Amendment No. 1

On April 1, 2003, Millennium modified non-clinical information (Amendment #012). The purpose of this amendment was to compare AUC₀₋₂₄ rather than AUC₀₋₇₂ hours at weeks 37 and 38. According to the sponsor, in the original submission the AUC data for these sampling

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intervals was expressed as AUC_{0-24 hr} when the data presented was for AUC_{0-72 hr}. This difference is not at all clear when the amendment is compared to the original submission. The new data submitted by the sponsor in the amendment are shown below. These changes affect the pharmacokinetics values (lower AUC than reported in the original submission) but do not impact the overall interpretation of the nonclinical PK data as it relates to human safety.

Note: Non GLP but QA statement is included in the Amendment report.

Summary Toxicokinetic Exposure Parameters for Weeks 1, 5, 37, and 38

Dose (mg/kg)	Week	Cycle/dose		Estimated C _{max} (ng/mL)	AUC ₀₋₂₄ (hr*ng/mL)	AUC ₀₋₂₄ /dose (hr*ng/mL/mg*kg)	
0.05	1	1/1	Mean	25.4	12.3	246	
			SD	10.9	2.69	53.8	
	5	2/4	Mean	45.3	45.1	902	
			SD	7.9	7.73	155	
	37	13/1	Mean	49.4	38.5	770	
			SD	9.18	5.56	111	
	38	13/3	Mean	48.2	45.4	909	
			SD	12.4	10.9	218	
	0.075	1	1/1	Mean	40.5	34.6	462
				SD	9.54	10.4	139
		5	2/4	Mean	82.3	82.9	1110
				SD	16.6	15.2	203
17		13/1	Mean	56.7	58.4	778	
			SD	18.7	13.8	180	
38		13/3	Mean	73.5	74.9	999	
			SD	13.6	17.8	237	
0.1		1	1/1	Mean	80.6	51.3	513
				SD	25.1	10.6	106
		5	2/4	Mean	138	111	1110
				SD	51.4	29.5	296
	37	13/1	Mean	81.7	72.8	728	
			SD	12.4	13.8	138	
	38	13/3	Mean	116	92.3	922	
			SD	14.5	14.3	143	

The new AUC₀₋₂₄ values for weeks 37 and 38 at 0.05, 0.075 and 0.1 mg/kg are approximately one half of the originally submitted values by the sponsor. AUC₀₋₂₄ increased in all dose groups at weeks 5, 37 and 38 as compared to week 1 indicating an accumulation of the drug in the plasma. The week 38 AUC₀₋₂₄ values are slightly higher/similar to the AUC₀₋₂₄ values at week 37 indicating that steady state levels of systemic exposure may have reached i.e., no accumulation after week 5. Examination of AUC_{0-∞} suggests possible accumulation to week 37.

PK/TK summary

The nonclinical PK, 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 as doses being evaluated in multiple myeloma (MM) patients. PS-341 is intended for IV administration in MM patients. Therefore, PK data generated after IV administration are summarized.

Absorption: The permeability properties of PS-341 were determined using Caco-2 cells. Good permeability in both the apical (A) to basolateral (B) and B to A directions indicated that PS-341 should permeate easily across cellular membranes. Similar flux values for both A to B and B to A directions suggest that PS-341 is unlikely to be a substrate for efflux pumps like Pgp.

Single and multiple doses IV PK of PS-341 were determined in rats and monkeys. The animals were dosed twice weekly followed by a week rest to mimic clinical dosing in cancer patients. Blood samples were collected after the first dose on weeks 1, 14 and 26. The C_{max} and AUC_{0-24} after repeated dosing were significantly higher as compared to week 1 suggesting accumulation of the drug. There were no additional increases in exposure at week 26 as compared to week 14 in rats indicating that steady state levels of systemic exposure may have reached.

Plasma protein binding and blood cell partitioning: The extent of binding of PS-341 to rat (85%), cynomolgus monkey (72%), and human (83%) plasma proteins was similar across the three species over the concentration range of _____.

Tissue distribution: _____

_____ were used to quantify [^{14}C]-PS-341 and its metabolites during 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. Peak concentrations occurred 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. The identity of the radioactivity remaining in the carcass is not known at this time.

Metabolism: PS-341 was extensively metabolized by rats, cynomolgus monkeys, and humans. More than 30 metabolites have been identified. These metabolites were formed by deboronation of PS-341 (mediated by cytochrome P450 (3A4 and 2D6) forming M1 and M2, followed by hydroxylation of the corresponding acids. Thus, PS-341 was primarily metabolized via CYP450 and not via Phase II pathways e.g. glucuronidation and sulfation. The major metabolites detected in rat bile were M1, M2, M10, M14, and M15. Metabolites M1, M2, and M15 were observed in monkey fecal samples after IV administration of PS-341. M1, M2 and M4 were the major metabolites detected in human plasma indicating resemblance of PS-341 metabolism in rodent, non-rodent and humans.

Inhibition and induction of metabolism: PS-341 was a poor inhibitor of recombinant human CYP P450 isozymes 1A2, 2C9, 2C19, 2D6, and 3A4 with IC_{50} values of $>18 \mu M$ ($\sim 7 \mu g/mL$). These IC_{50} values are higher than the observed PS-341 C_{max} concentration seen in cancer patients. Therefore, it is unlikely that PS-341 will change the metabolic clearance of concomitant medications. The potential increase or decrease in PS-341 activity by potent inducers or inhibitors of CYP3A4 and 2D6 has not been evaluated. Therefore, it is difficult to discuss drug-drug interaction with PS-341.

Elimination: Biliary excretion was the primary route of elimination of [^{14}C]-PS-341 in rats. In intact rats, 39% of the administered radioactivity was recovered in feces and 21% in urine. In bile duct-cannulated rats, 35% of the activity was recovered in bile, 16% in urine, and 8% in feces. Approximately, 32% of the administered radioactivity was found in the tissues and carcass after 72 hours post dose. In one male monkey, approximately 25% of the recovered radioactivity was excreted in the urine and 13% in the feces at 144 hours postdose. In a female monkey, urine and feces accounted for 11% and 15% of

the dosed radioactivity at 144 hours post dose, respectively. Selected tissues (GI tract, heart, kidneys, liver, lungs, and pancreas) and the carcass still contained 12 % and 19%, respectively, of the administered radioactivity at 144 hours postdose. The identity of the radioactivity is not known at this time. Plasma levels obtained from monkeys suggest possible enterohepatic recirculation. **It is ambiguous what the effects are, if any, of the retained radioactivity (PS-341 and/or metabolites).**

Pharmacokinetic and pharmacodynamic relationships: According to the sponsor, the PK/PD relationship in cynomolgus monkeys can be described by a simple maximum effect ($EC_{50} = 3.91$ ng/mL) model. At concentration >5 ng/mL proteasome inhibition appeared to reach a plateau (70-80%). In cancer patients, the PK/PD relationship is similarly described with EC_{50} of 1.48 ng/mL and a plateau of inhibition of proteasome at 2 ng/mL (DM98-194).

PK/TK conclusions

After single dose IV administration to rats and monkeys, plasma concentration of PS-341 decreased in a biphasic manner, a rapid distribution phase followed by a longer terminal elimination phase. The area under the plasma concentration-time curve (AUC) increased in a dose-dependent manner over the tested dosages (0.3-1.2 mg/m²). The elimination half-life in monkeys increased 3- to 4- fold after repeated dosing.

The PK of PS-341 cynomolgus monkeys and humans after single and multiple doses were similar as shown below.

Species	Cynomolgus Monkeys		Solid Tumor Patients	
	Week 1	Week 5	Cycle 1 Day 1	Cycle 1 Day 8
Dose (mg/m ²)	1.2	1.2	1.0	1.0
n	12	12	17	17
C _{max} (ng/mL) ^a	80.6 ± 25.1	138 ± 51.4	157 ± 134	126 ± 87.6
T _{1/2} (hr)	7.78 ± 3.16	9.68 ± 2.59	5.45 ± 4.50	19.7 ± 11.6
AUC ₀₋₂₄ (ng/mL*hr)	51.3 ± 10.6	111 ± 29.5	30.1 ± 15.3 ^b	54.0 ± 14.6
AUC (ng/mL*hr)	61.3 ± 14.0	140 ± 45.2	36.8 ± 18.3	81.9 ± 25.7

a: Estimated.

b: Median time for last quantifiable measurement was approximately 6.5 hours.

There was a decrease in clearance that produced an increase in the terminal elimination half-life and AUC after multiple dosing to cynomolgus monkeys. Similar findings were also observed in patients with solid tumors (Study RPT-00169) as shown above.

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Overview of Toxicokinetics Data

Parameter	Dose (mg/m ²)	Rat (time)	Cynomolgus Monkey (time)	Human (time)
Study #		PK800	PK 888	M34100-27
C _{max} (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)*	81±25(week 1), 116±15 (week 38)	
AUC ₀₋₂₄ (hr*ng/ml)	1.0			30.1±15.3 (cycle 1, day 1), 54.0±14.6 (cycle 1, day 8)
	1.2	65.3 (week 1), 163 (week 26)*	51±11 (week 1), 92±14 (week 38)	
Protein binding (%)		85	72	83

* High dose was reduced from 1.2 mg/m² to 0.9 mg/m² on study day 28/29 due to toxicity.

In conclusion, the PK of P-341 has been adequately characterized in rats and monkeys and can be compared with humans.

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IV. GENERAL TOXICOLOGY:

Reviewer name: Margaret E. Brower, Ph.D.

Preclinical toxicology overview:

A number of preclinical single-dose and multiple-dose studies have previously been submitted and reviewed. These include primarily studies in rodents and monkeys. Monkeys were administered PS-341 as a single dose, and for 24h, daily X13, twice weekly for 2weeks and twice weekly for 4weeks. The LD₁₀/HNSTD for multiple-dose studies ranged from 0.5-1.2mg/m²; common toxicities included lymphocytic depletion/necrosis of the lymph nodes, thymus and spleen, renal and hepatic necrosis, myocardial degeneration and GI toxicities. A cardiotoxicity study was conducted in monkeys at doses of 1.2-3.6mg/m²; doses \geq 3.0mg/m² were lethal, 2.4mg/m² produced tachycardia and moderate reversible hypotension and 1.2mg/m² produced no cardiotoxicity. In another study in monkeys assessing cardiovascular function, the left ventricular pressure of females administered 0.36mg/m² PS-341 exhibited a gradual increase of 20-40% above baseline over a 6h monitoring period; ventricular contractility fluctuated about \pm 40% over the same period.

Rodents were administered PS-341 as a single dose, weekly for 8 weeks and twice weekly for 2weeks (2 studies). The LD₁₀/HNSTD for multiple-dose studies ranged from 1.2-1.8mg/m². Findings included elevated ALT and AST, hepatic necrosis, and lesions of the kidneys, thymus, lymph nodes, colon and urinary bladder.

Previous studies support animal models, dose levels (HD of 1.2mg/m² for both species) and schedules of

9- and 38-three week studies conducted in rodents and monkeys, respectively, and reviewed below.

Observed clinical adverse events correlate with exhibited toxicities in preclinical studies, especially the monkey model, as shown below.

Multiple Dose Studies

Study Title: A 26-week intravenous injection toxicity study of PS-341 in the albino rat

Key study findings (See study summary on p6):

- Mortality at doses \geq 0.90mg/m² in 11/60 rodents
- Multifocal neurotoxicity
- Anemia and thrombocytopenia
- Lymphoid system debilitation
- Cardiac necrosis
- Chronic progressive nephropathy
- Non-linear HD toxicokinetic exposure

Project #: 57285

Volume #, and page #: Electronic submission: January 21, 2003, Module 4

Conducting laboratory and location: _____

Date of study initiation: November 21, 2001

GLP compliance: US GLP, OECD, Japanese GLP (with exception of toxicokinetic and pharmacodynamic data which were not conducted in compliance with GLP).

QA report: YES

Drug, lot # and % purity: PS-341 (3.5mg PS-341 and 35mg D-mannitol/vial), lot # D2-1-1, indicated to be stable for duration of study, drug purity not provided (test material reconstituted by adding 7mL of NaCl to vial to obtain a 0.5mg/mL stock solution which was diluted further using NaCl to obtain a 0.1mg/mL solution)

Formulation/vehicle: D-Mannitol (_____) purity _____, lot # 61112750/3800/04E27

Diluent: 0.9% NaCl, USP

Dosing:

Species/strain: Sprague Dawley [CrI:CD(SD) IGS BR]

#/sex/group + dose/group:

Dose (mg/kg) [mg/m ²]	Dose volume (mL/kg)	# of animals			
		Main study ^a		Toxicokinetic study	
		Males	Females	Males	Females
0	2/1.5 ^b	30	30	12	12
0.05 [0.3]	0.5	30	30	12	12
0.1 [0.6]	1	30	30	12	12
0.2/0.15[1.2/0.9 0] ^b	2/1.5 ^b	30	30	12	12

^a Animals from each treatment group (1-4) were assigned to 3 subgroups of 10 animals/sex; subgroups

were sacrificed following 14w of dosing, 26w of dosing, and following an 8w recovery period

^b Due to toxicity exhibited at 0.2mg/kg, dose reduced to 0.15mg/kg on study day 28/29. Due to an "oversight", dose volume was not reduced to reflect this dose change until day 45/46.

Note: Due to dosing error, one satellite control animal replaced. Due to technical error, one HD female overdosed by 43% on day 88. Study authors indicated that reaction of animal to treatment was comparable to other animals of group; therefore, error was considered to have no adverse impact on study results. Results will be reported below if toxicities appeared to differ for this animal.

Age: 6 weeks

Weight: males: 134-220g; females: 113-172g

Doses: see table above

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Route: iv, tail vein

Duration of dosing: 2X/week (72h between dosing periods) for 2w followed by 1w of rest; 3w cycle repeated until end of week 26 (9 cycles).

Results (Observation times)

Mortality (2X/day)	2/10 HD males and 4/10 HD females found dead or sacrificed moribund d5-73 (interim sac group) 2/20 HD males and 3/20 HD females found dead or sacrificed moribund d23-180 Total deaths: 4/30 HD males and 7/30 HD females(see Note below)
Clinical Observations (2X/day) [Daily complete physical examination]	Interim sac: Salivation, piloerection, yellow stained wet fur (see below for clinical observations for interim animals dead or sac moribund) All doses/final sac: Salivation, piloerection, soft feces, depressed fecal output from study week 5 to study termination MD, HD: decreased motor activity from study week 11 to study termination
Body weights (predose, weekly)	UR (10-12% depression at HD compared to concurrent controls at w26)
Food consumption (weekly)	UR
Ophthalmology (predose, w14, 26)	UR
Hematology (dose termination)	See table below [lymphocyte depression 40% in HD males at study termination]
Clinical chemistry (dose termination)	See table below 16% increase in glucose levels in LD/HD recovery males
Urinalysis (dose termination)	Dose-related increase in phosphorus levels in males (50% incr at HD)
Toxicokinetics (day 1, w14, w26: samples at 30', 1, 6, 24h) [Advion Biosciences, Ithaca NY for assessment]	See table below
Pharmacodynamics (day 1, w14, 26: samples at 30', 1, 6, 24, 48, 72h)	See results below following toxicokinetics
Organ weights (scheduled sacrifice at w26, 34 + unscheduled sacrifice)	See Histopathology Inventory for list of organs weighed Weights below compared to concurrent controls at 26 weeks: Liver: HD males ↑39%; LD (↑25%), MD (↑58%), HD (↑75%) females Thymus: HD males ↓37%; HD females ↓30% Kidney: MD female ↑18%, HD female ↑28%
Gross pathology (scheduled)	Liver, kidney, spleen: enlargement

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sacrifice at w26, 34 + unscheduled sacrifice)	Testes, epididymides: atrophy
Histopathology (scheduled sacrifice at w26, 34 + unscheduled sacrifice)	See Histopathology Inventory for list of tissues preserved and examined See table below for histopathological incidence

Note: Mortality or moribund sacrifice: HD males and 2/3 HD females exhibited decreased motor activity, hypersensitivity, non-sustained convulsions, salivation, hypothermia, closed eyes, piloerection, closed eyes with discharge, soft feces, dehydration, weakness, ungroomed fur and pallor. One male exhibited tremors, limited use of hindlimbs, swelling of hindlimbs and muzzle, vocalizing, inflammation of scrotum and thrombocytopenia. Clinical observations were prevalent to a greater extent and one animal died prior to dose reduction from 0.2 to 0.15mg/kg/dose. Microscopic findings included sciatic nerve necrosis (HD female), spinal cord inflammation (HD male), bone marrow necrosis/hypocellularity, gastric ulceration, Grade 1 endocardial/myocardial hemorrhage and necrosis in 2/2 animals, liver necrosis/pigment deposition/inflammation, necrosis of adrenal, lungs, kidney, heart, liver, GI tract, spleen, ovaries, thymus, lymph nodes and mammary glands.

Hematological indices at week 26^a

Parameter	Males (mg/kg)			Females (mg/kg)		
	0.05	0.1	0.2/0.1 5	0.05	0.1	0.2/0.1 5
RBC (10 ⁶ /uL)	UR	UR	↓23	UR	↓14	↓14
Hgb(g/dL)	UR	UR	↓20	UR	↓14	↓12
Hct (%)	UR	UR	↓16	UR	↓13	↓11
Platelets (10 ³ /uL)	↓15	↓45	↓57	↓11	↓31	↓49
Retics (%)	↑37	↑32	↑99	↑17	↑43	↑16

^a % compared to concurrent controls

Clinical chemistry indices at week 26^a

Parameter	Males (mg/kg)			Females (mg/kg)		
	0.05	0.1	0.2/0.1 5	0.05	0.1	0.2/0.15
AST (U/L)	↓36	↓43	↓53		↓20	↓24
ALT (U/L)	↓67	↓72	↓74	↓36	↓40	↓49
GGT (U/L)		↑80	↑400			↑188
BUN (mg/dl)			↑98	↓24	↓37	↓45
Glu (mg/dl)	↑17	↑26	↑56	↑17	↑38	↑72
Chol (mg/dl)	↓10	↓26	↓34		↑25	
Phos (mg/dl)			↑31	↑14		
Trig (mg/dl)						↑119

^a % compared to concurrent controls

Depressed clinical chemistry indices are of questionable biological significance.

Toxicokinetic parameters of rodents administered PS-341 (combined gender)

Dose (mg/kg)	AUC ₍₀₋₂₄₎ (hr.ng/mL) ^a			C _{max} (ng/mL)		
	Wk 1	Wk 14	Wk 26	Wk 1	Wk 14	Wk 26
0.05	14.5	84.6	84.7	1.5	5.4	4.7
0.10	27.4	145	134	3.3	10.7	10.9
0.2/0.15	65.3	137	163	8.9	17.2	22.6

^a Data amended by sponsor on April 3, 2003: Minor typographical errors were corrected in HD 1h timepoints in males and females at week 14. Summary data were not affected.

As with monkeys, there appeared to be no gender differences. A dose-dependent increase was exhibited in AUC and C_{max} at weeks 1, 14, and 26. Increases in AUC and C_{max} were also observed from week 1 to week 14 of administration; week 26 values were similar to those of week 14. The increase in AUC at the HD for weeks 14 and 26 was ~ one-half increased exposure at the lower doses at the same time periods when data were normalized. The explanation for non-linearity at the HD is unknown. Data should be considered general estimates as a result of only 4 timepoints measured following dosing at weeks 1, 14 and 26.

Pharmacodynamic evaluation generally exhibited a dose-dependent decrease in the mean 20S proteasome chymotryptic:tryptic ratio from 30m to 48h following dosing at weeks 1, 14 and 26. The maximum decrease in mean ratio activity was observed at 30m postdose; partial recovery of mean 20S ratio activities to predose levels was observed by 72h postdose.

Rodent histopathology data are presented in the following 6 tables. Table 1, 3, and 5 list findings and incidence of unscheduled deaths preceding interim sacrifice at week 14, final sacrifice at week 26 and recovery sacrifice. Tables 2, 4, and 6 list findings and incidence of animals sacrificed at scheduled time points. The study reports (separate study report for interim data) tabulated combined scheduled and unscheduled data, as well as these findings listed separately. As indicated in notes, comments and footnotes below, many of the histopathological findings were not mentioned in data assessed separately and there are some discrepancies in number of animals examined.

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Table 1. Histopathological findings in rodents administered PS-341 – Unscheduled sacrifice (d5-73)

Finding	Males (mg/kg)	Females (mg/kg)
	N=2 0.2/0.15	N=4 0.2/0.15
Adrenal/hypertrophy (Gr 1)		2
/necrosis (Gr 1)		1
Bone marrow/hypocellularity [males:Gr2/3; females Gr 3/4]	2	3
Heart/inflammation (Gr 3)	1	
/hemorrhage (Gr 1)	1	3
/necrosis (Gr 1)		2
Ileum/hyperplasia (Gr 1/2)	1	4
/necrosis (Gr 1/2)		3
Jejunum/hyperplasia (Gr 1-3)	1	4
/necrosis (Gr 1/2)	1	4
Liver/vacuolation (Gr 1/4)	2	3
/hypertrophy (Gr 2/3)	2	2
/necrosis (Gr 1/2)	1	4
/pigmented deposits (Gr 1)	1	
/increased mitotic activity (Gr 1)		1
/inflammation (Gr 2)		1
/reactive sinusoidal lining (Gr 2)		2
/hemorrhage (Gr 2)		1
Lung/necrosis (Gr 1/3)		3
Lymph node-mesenteric/necrosis (Gr 1/4)		2
Lymph node-mandibular/necrosis (Gr 1-4)	1	4
Lymph node-tracheobronchial/necrosis (Gr 1/3)		2
Mammary gland/necrosis (Gr 1)		2
Ovary/necrosis (Gr 1)		2
Rectum/hyperplasia (Gr 1/2)	1	4
/necrosis (Gr 3)		3
Salivary gland/hypertrophy (Gr 2)	2	2
/necrosis (1/2)	1	2
Spleen/necrosis (Gr 1/3)	2	4
/inflammation (Gr 2)	1	

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Stomach/necrosis (Gr 1/2)		2
Thymus/necrosis (Gr 1-4)	2	4
Nasal cavity/necrosis (Gr 1/3)		2
Cecum/hyperplasia (Gr 1/2)		3
/necrosis (Gr 1)		2
Colon/hyperplasia (Gr 2/3)		3
/necrosis (Gr 1)		2
Duodenum/hyperplasia (Gr 1/2)		4
/necrosis		3
Kidney/degeneration (Gr 3)		1
/necrosis (Gr 1)		2
Lacrimal gland/atrophy (Gr 2)		2

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Females sacrificed prior to schedule exhibit greater degree of toxicity as compared to males.

Table 2. Histopathological findings in rodents administered PS-341 Interim sacrifice (wk 14)

Finding	Males (mg/kg)			Females (mg/kg)		
	0.05	0.1	0.2/0.1 5	0.05	0.1	0.2/0.1 5
Cecum/hyperplasia (Gr 1/2)		4/10	4/8			4/6
Colon/hyperplasia (Gr 1/2)		3/10	5/8		3/10	3/6
Duodenum/hyperplasia (Gr 1/2)		10/10	8/8		7/10	6/6
Heart /inflammation ^d	1/10					
/hemorrhage ^d	1/10					
/mononuclear c. infiltration ^{a,d}	6/10	3/10	2/8	3/10		
Jejunum/hyperplasia (Gr 1/2)		8/10	8/8			6/6
Ileum/hyperplasia (Gr 1/2)		5/10	8/8			6/6
Kidney /chronic progressive nephropathy ^d					2/10	1/6
Lacrimal gland/necrosis (Gr 2)			1/8			
Liver/ vacuolation (Gr 1/2)		1/10	2/8	2/10	6/10	5/6
/hypertrophy (Gr 1/2)	4/10	6/10	5/8	9/10	10/10	5/6
/pigmented deposits (Gr 1)	2/10	4/10			2/10	2/6
/inflammation ^d				5/10		
Lung/histiocytosis ^d				1/10		
Lymph node/hyperplasia		1/10				
/necrosis		3/10	1/8			1/6
/congestion ^d		1/10	4/8		1/10	1/6
Mammary gland/adenoma ^d					1/10	
Cervical dorsal root ganglia/degeneration ^d						1/6

Cervical ventral root/degeneration ^d						1/6
Lumbar dorsal root ganglia/degeneration ^d						1/6
Thoracic dorsal root ganglia/degeneration ^d			2/8			
Peroneal nerve/degeneration ^d						1/6
Sural nerve/degeneration ^d						1/6
Sciatic nerve/degeneration ^{bd}			6/8			4/6
Spinal cord/degeneration ^{cd}						6/6
Rectum/hyperplasia (Gr 1/2)			3/8		1/10	6/6
/necrosis (Gr 1/2)			1/8		1/10	3/6
Salivary gland/hypertrophy (Gr 1/2)		3/10	6/8		2/10	5/6
Thymus/necrosis (Gr 1-4)			7/8			4/6

^a Incidence in control males: mononuclear c. infiltration, 2/10

^b Incidence in control males: 5/10; control females: 5/10

^c Incidence in control females: 7/10

^d Findings only listed in table combining histopathological findings in decedents and scheduled sacrifice; findings not listed in tables listing decedents separately from scheduled sacrifice

Degeneration of peripheral nerves was similar in dosed and control animals when not individually listed

Comment: A number of findings were only listed in histopathology table combining interim decedents and scheduled sacrifice, including all neurotoxic findings

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Table 3. Histopathological findings in rodents administered PS-341 – Unscheduled sacrifice (d23-180)

Note: Study report indicated unscheduled deaths in 3 females between d23 and d180; only 2 females examined histopathologically in table designated week 26, third animal in separate table designated recovery

Finding	Males (mg/kg) N=2	Females (mg/kg) N=2
	0.2/0.15	0.2/0.15
Adrenal/vacuolation (Gr 2)		2
/necrosis (Gr 1)		1
Bone marrow/hypocellularity Gr 3		1
Heart/inflammation (Gr 1)	1	
/hemorrhage (Gr 1)		2
/necrosis (Gr 1)		2
Ileum/hyperplasia (Gr 2)	1	2
/necrosis (Gr 1/3)		2
Jejunum/hyperplasia (Gr 1/2)	1	2
/necrosis (Gr 3)		2
Liver/vacuolation (Gr 3)	1	2
/hyperplasia (Gr 2/3)		1
/necrosis (Gr 1/2)	1	2
/pigmented deposits (Gr 1)	1	1
/inflammation (Gr 2)	1	
Lung/necrosis (Gr 1/2)	1	2
/inflammation (Gr 1/2)	1	2
Lymph node-mesenteric/necrosis (Gr 1/3 males; Gr 2/3 females)	2	2
Lymph node-mandibular/necrosis (Gr 1 males; Gr 2/3 females)	1	2
Lymph node-tracheobronchial/necrosis (Gr 1/2)	1	2
Mammary gland/necrosis (Gr 1)		1
Ovary/necrosis (Gr 1/2)		2
Rectum/hyperplasia (Gr 1/2)	1	2
/necrosis (Gr 2)		2
Salivary gland/hypertrophy (Gr 1/2)	1	2
/necrosis (1)		1
Sciatic nerve/necrosis (Gr 1)		1
Spinal cord/inflammation (Gr 1)	1	
Spleen/necrosis (Gr 2/3)m	2	2
/inflammation (Gr 2)	1	
/histiocytosis (Gr 2/3)	1	2
Stomach/ulceration (Gr 2)m	1	
Thymus/necrosis (Gr 1-3)m	2	2
Nasal cavity/necrosis (Gr 1/3)		2
Cecum/hyperplasia (Gr 1/2)	1	2

/necrosis (Gr 3)		1
Colon/hyperplasia (Gr 2/3)	1	2
/necrosis (Gr 1/2)		2
Duodenum hyperplasia (Gr 2)	1	2
Kidney degeneration (Gr 1/2)	1	2
/inflammation (Gr 3)	1	
Lacrimal gland/atrophy (Gr 2)		1

Females sacrificed prior to schedule exhibit greater degree of toxicity as compared to males.

Table 4. Histopathological findings in rodents administered PS-341 Terminal sacrifice (wk 26)

Note: Study report indicated unscheduled deaths in 2 HD males between d23 and d180; histopathological findings were listed separately for these animals (table above); however, tabulated findings for males sacrificed at w26 listed number examined at HD as N=10, animals dead on study indicated in separate recovery table

Finding	Males (mg/kg)			Females (mg/kg)		
	0.05	0.1	0.2/0.1 5	0.05	0.1	0.2/0.1 5
Bone marrow/hypocellularity		1/10	6/10			2/8
Brain/gliosis, dilatation			1/10			1/8 ^B
Lumbar dorsal root ganglia/degeneration ^b			2/10			
Lumbar dorsal root/degeneration						4/8 ^B
Lumbar ventral root/degeneration						1/8 ^B
Cervical ventral nerve root/degeneration			1/10			
Peronal nerve/degeneration			1/10			1/8 ^B
Sciatic nerve/degeneration ^c			8/10			4/8 ^B
Thoracic dorsal root/degeneration			1/10			
Spinal cord/degeneration ^d						3/8 ^B
Cecum/hyperplasia		4/10	9/10	3/10	2/10	4/8
Colon/hyperplasia		4/10	9/10		3/10	1/8
Duodenum/hyperplasia		4/10	10/10		5/10	2/8
Ileum/hyperplasia		3/10	9/10		4/10	4/8
/necrosis		1/10	7/10			
Jejunum/hyperplasia		3/10	9/10		4/10	2/8
/necrosis		1/10	5/10			
Kidney/necrosis			5/10			
/chronic progressive nephropathy ^e	7/10	6/10	4/10	2/10	2/10	
/eosinophilic casts					3/10	6/8
/inflammation					1/10	3/8

/hypertrophy					6/10	7/8
/tubular dilatation		3/10	8/10		1/10	3/8
/glomerulonephritis			2/10			
Lacrimal gland/necrosis		1/10	2/10			
Liver/hypertrophy	4/10	10/10	7/10	10/10	10/10	8/8
/hepatocellular vacuolation				4/10	7/10	8/8
/pigment deposits				5/10	7/10	6/8
Lung/necrosis			3/10			
Mandibular lymph node/atrophy			6/10			1/8
Mesenteric lymph node/atrophy		7/10	10/10		5/10	3/8
Tracheobronchial L.N./atrophy		2/10	5/10		1/6	2/6
Prostate/necrosis			3/10			
Rectum/necrosis		1/10	6/10			
/hyperplasia		4/10	9/10		1/10	2/8
Seminal vesicle/necrosis		1/10	4/10			
Spleen/necrosis		7/10	7/10		1/10	2/8
Thymus/necrosis		3/10	9/10		8/10	8/8
Heart/necrosis, perivascular (Gr1)			1/10			
/degeneration, necrosis, myocardial ^e		1/10		1/10	2/10	1/8 ^g
/hemorrhage						2/8 ^g
/inflammation, myocardial, epicardial			1/10			
/mononuclear cell infiltration ^f	6/10	1/10	2/10	2/10		1/8 ^g
Ovary/necrosis				3/10	4/10	5/8
Salivary gland/hypertrophy				4/10	4/10	8/8

^a Incidence 5/10 in control males, 2/10 in control females

^b Lumbar dorsal root, dorsal root ganglia, ventral root, spinal cord, thoracic ventral root and cervical dorsal root degeneration in control (1-3/10) as well as HD males (1-3/10)

^c Incidence 8/10 in control males; 3/10 in control females

^d Cervical, lumbar, thoracic spinal cord: Incidence 1/10 in control females (cervical), 2/10 (thoracic)

^e Incidence 2/10 in control males, 1/10 in control females

^f Incidence 6/10 in control males, 1/10 in control females

^g Findings only listed in table combining histopathological incidence in decedents and scheduled sacrifice; findings not indicated in table of listing decedent females separately from scheduled sacrifice

If not specified in above table, severity of cardiac lesions was not reported.

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Table 5. Designated preterminal sacrifice - recovery period (sac at 26w)

Finding	Females (mg/kg)
	N=1
	0.2/0.15
Adrenal/hypertrophy (Gr 2)	1
Bone marrow/hypocellularity Gr 3	1
Ileum/hyperplasia (Gr 1)	1
/necrosis (Gr 1)	1
Jejunum/hyperplasia (Gr 1)	1
/necrosis (Gr 2)	1
Liver/vacuolation (Gr 3)	1
/necrosis (Gr 2)	1
Lung/necrosis (Gr 1)	1
Lymph node-mesenteric/necrosis Gr 2	1
Lymph node-mandibular/necrosis Gr 1	1
Salivary gland/hypertrophy (Gr 2)	1
Spleen/necrosis (Gr 2)	1
/histiocytosis (Gr 1)	1
Thymus/necrosis (Gr 2)	1
Cecum/hyperplasia (Gr 1)	1
/necrosis (Gr 1)	1
Colon/hyperplasia (Gr 1)	1
Duodenum/hyperplasia (Gr 2)	1
/necrosis (Gr 1)	1

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Table 6. Histopathological findings in rodents administered PS-341 Recovery sacrifice
 Note: Number of HD males examined listed as 8, presuming 2 deaths or moribund sacrifice. The histopathological findings of these animals were listed for unscheduled sacrifice week 26 (tabulated above). Designated as 1/10 unscheduled HD female deaths (table above).

Finding	Males (mg/kg)			Females (mg/kg)		
	0.05	0.1	0.2/0.1 5	0.05	0.1	0.2/0.1 5
Bone marrow/hypocellularity			2/8			2/8
Optic nerve/atrophy			1/3 ^b			
Sciatic nerve/degeneration			2/2 ^b			
Tibial nerve/degeneration			1/2 ^b			
Spinal cord/degeneration			1/2 ^b			
Nasal cavity/inflammation of nasolacrimal duct			2/8			
Heart/epicardial, endocardial inflammation		1/10	1/8 ^b			
/myocardial inflammation ^c	3/10	1/10	4/8 ^b			1/9 ^b
/degeneration, necrosis ^f	2/10	4/10	2/8 ^b	1/10	1/10	
/mononuclear cell infiltration ^g	3/10	4/10	3/8 ^b	1/10	2/10	1/9 ^b
/vascular inflammation				1/10		
Kidney/chronic progressive nephropathy ^a	7/10	4/10	7/8 ^b	4/10	4/10	4/9 ^b
/tubular dilatation		4/10	7/8			
Liver/vacuolation ^b	6/10	2/10	2/8	1/10	1/10	
/tension lipidosis ^c				4/10	6/10	4/9 ^b
/pigment						8/9
Lymph nodes/necrosis ^d			2/8 ^b			1/9 ^b
Pancreas/necrosis			1/8			
Pituitary/adenoma			1/3 ^b			
Spleen/atrophy			2/8			
/increased pigment deposit				5/10	8/10	9/9
Testes/atrophy			1/8			
Thymus/atrophy			1/8			1/9 ^b

^a Incidence in control males 4/10, 3/10 control females

^b Incidence in control males 2/10

^c Incidence in control males 3/10

^d Mandibular, mesenteric, tracheobronchial in males; mesenteric, mandibular in females

^e Incidence in control males 2/10, 1/10 control females

^f Incidence in control males 3/10, 1/10 control females

^g Incidence in control males 2/10, 4/10 control females

^h Findings not indicated in recovery table separating prescheduled termination from animals sacrificed at scheduled recovery

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Severity of cardiac lesions was not reported.

Study Conclusions:

Mortality at doses $\geq 0.90\text{mg}/\text{m}^2$ (11/60 rodents) was due primarily to hematopoietic, gastrointestinal and lymphoid system debilitation; similar findings were observed in scheduled deaths. Female decedents 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. Reduced motor activity was observed at $\geq 0.6\text{mg}/\text{m}^2$. Neurotoxicity was multifocal and included brain dilatation, and degeneration of dorsal root ganglia, peripheral nerves and spinal cord; neurotoxicity was limited to HD rodents. Thrombocytopenia was observed at all dose levels and anemia was exhibited in HD males and MD and HD females. Depressed thymus weights, accompanied by lymphocyte depletion, were observed at the HD. Chronic progressive nephropathy was generally observed at 26w and following recovery; males appeared to be more susceptible to kidney changes. Lymphoid atrophy and/or necrosis were exhibited in thymus, spleen and lymph nodes. Histopathological changes in cardiac tissue were comparable to control animals with few exceptions (slight increased incidence of perivascular necrosis, degeneration, hemorrhage and inflammation) at the MD and HD. Following recovery, myocardial and vascular inflammation, and cardiac necrosis was observed sporadically at all doses; the incidence of findings was not dose dependent. The severity of cardiac findings was not reported; therefore, the effect of PS-341 on rodent cardiotoxicity could not be clearly determined. Following the 8-week recovery period, there appeared to be some indication of reversibility of findings observed during dosing. Non-linearity of exposure with increasing dose was observed at the HD; the explanation for non-linearity is unknown.

Study Title: A 38-week (13-cycle) intravenous injection toxicity study of PS-341 in the cynomolgus monkey

Key study findings (See study summary on p 12) :

- Mortality at 0.9 and 1.2mg/m² in 2/13 animals
- Anemia and thrombocytopenia
- Multifocal neurotoxicity
- Cardiac tissue necrosis, inflammation and hemorrhage
- Glomerulonephropathy
- Lymphoid atrophy
- GI toxicity

Project #: 57284

Volume #, and page #: Electronic submission: January 21, 2003, Module 4

Conducting laboratory and location: _____

Date of study initiation: January 3, 2002

GLP compliance: US GLP, Japanese GLP (with exception of toxicokinetic, pharmacodynamic data and viral screen which were not conducted in compliance with GLP)

QA report: YES

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Drug, lot # and % purity: PS-341 (3.5mg PS-341 and 35mg D-mannitol/vial), lot # D2-1-1, indicated to be stable for duration of study, drug purity not provided

Fermentation/vehicle: D-Mannitol

purity 96%, lot # 61112750/3800/04E27CA
Diluent: 0.9% NaCl, USP

Dosing:

Species/strain: Cynomolgus monkeys

#/sex/group^a + dose/group:

Dose (mg/kg) [mg/m ²]	Dose volume (mL/kg)	^b # of animals			
		Main study		Recovery	
		Males	Females	Males	Females
0	1.5	3	3	3	3
0.050 [0.6]	0.75	3	2	-	1
0.075 [0.9]	1.13	3	3	-	-
0.100 [1.2]	1.5	3	4	3	2

^a Total animals dosed: 3/sex/group for LD and MD; 6/sex for HD; 6/sex for D-mannitol vehicle control

^b 3 monkeys/sex in control and HD groups only were scheduled to be retained for 8-week recovery. Due to toxicities observed 1 LD female also retained on recovery. Due to moribund sacrifice of 2HD females during study, only 2HD females retained for recovery

Note: 1 of 3 HD recovery males administered "small amount" of PS-341 subcutaneously; 1 of 3 control recovery males underdosed

Age: N/A

Weight: males: 1.7-2.5kg; females: 1.7-2.4kg

Doses: see table above

Route: iv via saphenous or brachial vein

Duration of dosing: 13 three-week cycles (cycle of dosing: twice weekly administration for 2w followed by 1wk rest)

Recovery period: 8weeks

Results (Observation times)

Mortality (2X/day)	Moribund sacrifice: 1 of 3 MD F (d179, cycle 9) result of severe anemia, thrombocytopenia; 1 of 6 HD M (d50, cycle 3 result of severe anemia, thrombocytopenia; 2 of 6 HD F (d172, 197, cycles 9 and 10) result of severe GI intolerance with dehydration (1 of 6), severe anemia, thrombocytopenia (1 of 6) [see note below]
Clinical Observations (2X/day) [Weekly detailed physical examination]	LD: Alopecia, soft feces, reddened skin MD: Tremors (1/3M, 1/3F), alopecia, reddened, dry skin, decreased motor activity, emaciation, soft feces, emesis, hunched posture, labored respiration HD: Alopecia, reddened or discolored, dry skin, soft feces,

	salivation, decreased motor activity, emesis, prostration, hunched posture Dose-related increase in frequency of observations
Neurological examination (2h following dosing w5, 11, 26, 38, including assessment of cranial and spinal nerves)	Pupillary light reflex (eye reaction): abnormal in 1/3LD F Flexor reflex (base of nail/each limb pinched w/ hemostat): no reaction in 1/3MD M, 1/3MD F, 1/6HD F Perineal reflex (anus stimulated w/hemostat; normal reaction= anal sphincter contraction, tail flex): no reaction in 1/3LD M, 1/3LD F, 1/3MD M, 1/3MD F, 1C Nasal septum test (nasal septum pinched w/forceps): no reaction in 1/3LD F, 1/3MD F, 2/3MD M, 1/6HD M
Body weights (d-1, weekly)	Animals sacrificed moribund: 17-56% depression in BW UR in remaining animals
Food consumption (daily)	Animals sacrificed moribund: significantly depressed UR in remaining animals
Ophthalmology (predose, w 8,37)	UR
EKG (predose, following dosing d4, w5, 38)	UR
Hematology (predose, w2, 4, 11, 25, 32, 35, 36, 37) ^{a,b}	LD, MD, HD:Dose-dependent depression of RBC, hgb, hct and platelet counts and increased reticulocyte counts. Recovery of parameters following 8wk (see table below)
Clinical chemistry (predose, w2, 4, 11, 25)	ALT(% change compared to concurrent controls): MD M: incr 42-46% wks10 and 24, 50% wk 38 HD M: incr 67% wk3, 135% wk10, 32-39% wks 24 and 38 HD F: incr 108% wk10, 80% wk24 Other changes UR
Urinalysis (predose, w2, 4, 11, 25)	MD, HD (M+F): decreased urine pH compared to concurrent controls; moderate to significant blood in urine Recovery HD F: moderate blood in urine [histological correlation w/ intratubular hemorrhage/kidney]
Toxicokinetics (predose, 10', 30', 1, 6, 24h postdose on d1, w5, 37, 38 + 48, 72h postdose w37, 38)	See table below
Pharmacodynamics (predose, 10', 30', 1, 6, 24, 48, 72h on d1, w5, 37, 38)	See results below following toxicokinetics
Organ weights (scheduled sacrifice d264, 320 + unscheduled sacrifice)	See Histopathology Inventory for organs weighed Changes in absolute weights as compared to concurrent controls: Main study:

	<p>Liver: increase in MD (26%) and HD (57%) M and HD (42%) F Kidney: increase in LD(16%), MD (41%) and HD (75%) M and MD (36%) and HD (61%) F [histological correlation w/ hyperplasia and inflammation/liver and tubular degeneration of kidney]</p> <p>Recovery study Spleen: increase in HD (73%) M and (46%) F Lymph node: increase in HD (35%) M and (22%) F [histological correlation w/ compensatory lymphoid hyperplasia]</p>
Gross pathology (scheduled sacrifice d264, 320 + unscheduled sacrifice)	<p>Kidney: dark foci, pale foci – all dosed M, HD F Spleen: raised areas – LD, HD recovery F</p>
Histopathology (scheduled sacrifice d264, 320 + unscheduled sacrifice)	<p>See Histopathology Inventory for complete list of tissues preserved; full histopathological assessment conducted on non-recovery animals and animals sacrificed moribund. Histopathology limited to target tissues (peripheral nerves including sciatic, trigeminal, peroneal, sural, tibial, skeletal muscles, spinal cord for recovery animals. See table below for incidence of findings by dose group</p>

^a Flow cytometry analysis for total T-cells, helper T-cells, cytotoxic T-cells, natural killer cells, B-cells and lymphocytes conducted on blood samples collected d250, 253, 260, 261, 264, 289, 318.

^b Viral screen (Simian retrovirus) conducted on 1 control and HD female monkey to investigate cause of decreased RBC and platelets; animals tested negative for presence of the virus.

Note: Moribund sacrifice: Histopathological neurotoxic findings documented in animals sacrificed due to anemia (MD, HD) were multifocal and targeted to dorsal root ganglia, peripheral nerves and spinal cord. Findings included swelling, degeneration axons and myelin, myelinophages, and foci of reactive gliosis. Animals sacrificed due to anemia exhibited the following: depressed RBC (78-93%), Hgb (73-90%), Hct (75-90%) and platelets (82-93%).

Toxicokinetic parameters of monkeys administered PS-341

Dose (mg/kg)	AUC ₍₀₋₂₄₎ (hr.ng/mL)				C _{max} (ng/mL)			
	Wk 1	Wk 5	Wk 37 ^a	Wk 38 ^a	Wk 1	Wk 5	Wk 37	Wk 38
0.050	12.3	45.1	39	45	25.4	45.3	49.4	48.2
0.075	34.6	82.9	58	75	40.5	82.3	56.7	73.5
0.10	51.3	111	73	92	80.6	138	81.7	116

^a Data amended by sponsor on April 3, 2003: AUC data submitted in original study report was incorrectly reported as AUC₍₀₋₂₄₎ rather than actual sampling interval of AUC₀₋₇₂. AUC₍₀₋₂₄₎ values tabulated above for wk 37 have been changed from 67, 108, and 138hr.ng/mL; values

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tabulated for wk 38 have been changed from 83, 140 and 170hr.ng/mL, for the LD, MD and HD, respectively.

There appeared to be no gender differences. A dose-dependent increase in C_{max} and AUC was exhibited. An increase in C_{max} was also found following repeat administration from weeks 1 to 5. Exposure observed at week 5 were ~2 to 3-fold higher than exposure at week 1, indicating drug accumulation. It is this reviewers' opinion that amended exposure data for weeks 37 and 38 appear to be based on estimation in order to enable direct comparison of AUC at weeks 1, 5, 37 and 38. Toxicokinetic data should be considered general estimates based on low numbers of animals and only 5-6 timepoints measured following dosing. [Refer to the Pharmacokinetics section of this document for additional discussion.] PS-341 appears to have reached steady state at week 5 and remained at steady state to week 38. Decreased exposure at week 37 and increased exposure at week 38 may be a reflection of the above factors. Drug exposure with increasing dose was more linear in monkeys than rodents. Pharmacodynamic evaluation exhibited a dose-dependent decrease in the mean 20S proteasome chymotryptic:tryptic ratio at weeks 1, 5 and 37. The maximum decrease in mean ratio activity was observed at 10 and 30m postdose; recovery of mean 20S ratio activities to predose levels was observed 24-48h postdose.

Hematological indices at d264 (24h following final dose administration)^a

Parameter	Males (mg/kg)		Females (mg/kg)		
	0.075 ^b	0.10	0.05	0.075	0.10
RBC (10 ⁶ /uL)	UR	↓23	↓19	UR	↓12
Hgb (g/dL)	↓13	↓23	↓18	UR	↓13
Hct (%)	↓11	↓21	↓17	UR	↓12
Platelets (10 ³ /uL)	↓47	↓70	↓65	↓33	↓68

^a % compared to concurrent controls (does not include animals sacrificed moribund prior to scheduled preterminal sacrifice)

^b Indices of LD males UR compared to concurrent controls

All above parameters appeared normalized following the 8-week recovery period with few exceptions (eg. reticulocyte counts remained elevated in sporadic animals)

Note:

The sponsor was requested by Pharm/tox to apply improved staining technique to CNS and peripheral nerve tissue.

Study histopathology notes indicate the following:

1. Sciatic nerves were embedded in _____, and embedded in _____ sectioned at 0.5-1um, and stained with _____ prior to examination.
2. Trigeminal, peroneal, sural, tibial nerves and spinal cord (including dorsal and ventral roots with corresponding dorsal root ganglion) were embedded in _____ and stained with _____. Dorsal and ventral roots with corresponding dorsal root ganglion were also embedded in _____ and stained with _____

3. For each non-recovery animal, dorsal and ventral roots, with corresponding dorsal root ganglia from both sides of cervical (C1 to C4), thoracic (T5 to T8) and lumbar (L4 to L7) spinal cord segments were embedded in _____ and stained by the _____ technique for the appearance of apoptotic cells.
4. For all animals, tissue samples from sciatic nerves were fixed in glutaraldehyde and paraformaldehyde for 24h and stored in formalin; these samples were not processed.

Histopathological results of CNS and peripheral nerves were presented in incidence tables combined with other tissue data (see below). Description of neuropathology indicated detailed observation of peripheral nerves and myelin. _____ results of spinal cord tissue were not described. Electron microscopy was not conducted.

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Histopathological findings in monkeys administered PS-341- Unscheduled sacrifice

Finding	Males (mg/kg)			Females (mg/kg)		
	0.05	0.075	0.10	0.05	0.075	0.10
Bone marrow/hypocellularity Gr1-2			1/1		1/1	2/2
Brain/hemorrhage			1/1			
/granular cell loss			1/1			
/necrosis Gr3					1/1	
Lumbar dorsal root ganglia/degeneration Gr2			1/1		1/1	2/2
/degeneration Gr1			1/1			
Peroneal nerve/degeneration			1/1			1/2
Sciatic nerve/degeneration			1/1			2/2
Tibial nerve/degeneration			1/1			1/2
Sural nerve/degeneration						1/2
Spinal cord/degeneration			1/1		1/1	2/2
Eye/retinal hemorrhage			1/1		1/1	1/1
Eye/degeneration					1/1	1/2
Kidney/inflammation Gr3, hyaline casts			1/1		1/1	1/2
/degeneration, hypertrophy Gr3			1/1			1/2
/glomerulonephropathy			1/1		1/1	
Liver/degeneration, necrosis			1/1		1/1	1/2
Spleen, thymus, adrenal/atrophy			1/1		1/1	2/2
Cecum/hyperplasia					1/1	2/2
Duodenum, ileum, jejunum, colon, rectum /hyperplasia						1/2
Heart/degeneration, necrosis					1/1	1/2
/inflammation						1/2
/hemorrhage					1/1	1/2
/mononuclear cell infiltration			1/1			1/2
Lymph node/atrophy, necrosis					1/1	1/2

Severity of cardiac lesions was not reported.

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Histopathological findings in monkeys administered PS-341- Terminal sacrifice

Finding	Males (mg/kg)			Females (mg/kg)		
	0.05	0.075	0.10	0.05	0.075	0.10
Bone marrow/hypocellularity	1/3		1/2			1/2
Cervical dorsal root/degeneration nerve			2/2		1/2	
Cervical ventral root/degeneration nerve	1/3	1/3				
Lumbar dorsal root/degeneration nerve		1/3	1/2	1/2		1/2
Lumbar ventral root/degeneration						2/2
Peroneal nerve/degeneration		2/3	2/2			
Meninges/hemorrhage		2/3				1/1
Sciatic nerve/degeneration		1/3	1/2	2/2	2/2	1/2
Sural nerve/degeneration		1/3				1/2
Tibial nerve/degeneration		1/3	1/2			1/2
Spinal cord/degeneration		1/3	1/2			2/2
Thor. ventral root/degeneration nerve						1/2
Kidney/degeneration, hypertrophy		3/3	2/2			1/2
/glomerulonephropathy		1/3	2/2			2/2
/inflammation	1/3	3/3	2/2		1/2	1/2
/hyaline cast		3/3	1/2			1/2
Colon, cecum, duodenum, ileum, jejunum/hyperplasia		1/3	2/2			
Cecum/necrosis						1/2
Rectum/atrophy			1/2			1/2
Spleen/atrophy, single c. necrosis		1/3	1/2			
Thymus/atrophy		1/3	2/2	2/2		2/2
Esophagus, gall bladder/inflammation	1/3		1/2			
Heart/degeneration, necrosis		1/3				
/inflammation		1/3				
/mononuclear cell infiltration ^a	1/3	1/3	1/2	2/2	1/2	1/2
Injection site/hemorrhage, inflammation	3/3	3/3	2/2			
Injection site/hemorrhage				1/2	2/2	1/2
Liver/leukocytosis		2/3	1/2			
/hyperplasia, inflammation			1/2			
/granuloma (parasitic)						1/2
Lung/fibrosis, inflammation			1/2			
/granuloma		1/3				
Trachea/inflammation			1/2			
Lymph node (axillary)/atrophy		1/3	2/2		1/2	
Lymph node (mandibular)/atrophy			2/2		1/2	2/2

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Lymph node (mesenteric)/atrophy		1/3	1/2		1/2	2/2
Lymph node (tracheobronchial)/atrophy		2/3	2/2		1/2	1/2

^a Incidence in controls: males 2/3, females 1/3

Severity of cardiac lesions was not reported.

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Histopathological findings in monkeys administered PS-341- Recovery sacrifice

Finding	Males (mg/kg)	Females (mg/kg)	
		0.10	0.05 0.10
Bone marrow/hypercellularity	2/3	1/1	
Cervical dorsal root/degeneration nerve	1/3		
Lumbar dorsal root/degeneration nerve	3/3	1/1	2/2
Lumbar ventral root/degeneration	2/3	1/1	2/2
Sciatic nerve/degeneration	2/3		2/2
Spinal cord/degeneration	1/3		2/2
Skeletal muscle/degeneration	1/3		
Heart/mononuclear cell infiltration ^a	3/3		2/2
Kidney/glomerulonephropathy	1/3		2/2
/tubular regeneration			1/2
/hyaline cast			1/2
Cecum, colon/hemorrhage	1/3		
Lymph node(mandibular)/hyperplasia	2/3	1/1	1/2
Thymus/atrophy	1/3		1/2
Spleen/hyperplasia		1/1	1/2

^a Incidence in controls: males 1/3, females 1/3

Changes in peripheral nerves and spinal cord indicated swollen and degenerating axons with secondary myelin breakdown, which appeared to be indicative of peripheral neuropathy.

Study Conclusions:

Mortality at 0.9mg/m² (1/6) and 1.2mg/m² (2/7 [main study animals]) due to severe anemia, thrombocytopenia and GI intolerance with dehydration; neurotoxicity was multifocal and included brain necrosis and swelling and degeneration of axons and myelin of dorsal root

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ganglia, peripheral nerves and spinal cord. Histopathological changes in cardiac tissue were minimal and were not dose dependent. Evidence of lack of neurological reflex at multiple sites at all dose levels. Anemia and severe thrombocytopenia were prevalent. Blood in urine at MD and HD. C_{max} and AUC increased in a dose-dependent manner; drug accumulation was observed at week 5 and remained at steady state to week 38. Animals sacrificed following scheduled dose administration also exhibited multifocal nerve degeneration of dorsal root ganglia, peripheral nerves and spinal cord at all doses; the incidence of neurotoxicity was reduced following recovery. Glomerulonephropathy was observed in males and females; 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. Drug exposure was more linear in monkeys compared to rodents; the explanation for this is not known.

Toxicology Summary:

Monkeys were administered PS-341 as a single dose, for 24h, daily X13, twice weekly for 2w, twice weekly for 4w and for 13-three week cycles. The 13-cycle study is discussed in detail below. The $LD_{10}/HNSTD$ for multiple-dose studies ranged from 0.5-1.2mg/m²; common toxicities included lymphocytic depletion/necrosis of the lymph nodes, thymus and spleen, renal and hepatic necrosis (without significant change in liver function test indices), myocardial degeneration and GI toxicities. A cardiotoxicity study was conducted in monkeys at doses of 1.2-3.6mg/m²; doses ≥ 3.0 mg/m² were lethal, 2.4mg/m² produced tachycardia and moderate reversible hypotension and 1.2mg/m² produced no cardiotoxicity.

When monkeys were dosed for 13-three week cycles of twice weekly dosing for 2w followed by 1w rest, mortality was observed at 0.9 and 1.2mg/m² in 3/13 animals. Mortality was attributed to severe anemia, thrombocytopenia and GI intolerance with dehydration; neurotoxicity was multifocal and included brain necrosis and swelling and degeneration of axons and myelin of dorsal root ganglia, peripheral nerves and spinal cord. Histopathological changes in cardiac tissue were minimal and were not dose dependent; however, the severity of cardiac findings was not reported. Evidence of lack of neurological reflex was observed at multiple sites at all dose levels. Anemia and severe thrombocytopenia were prevalent. Blood was observed in the urine of animals administered 0.9 and 1.2mg/m² PS-341 which correlates with intratubular hemorrhage observed in the kidney. C_{max} and AUC increase was dose-dependent; drug accumulation was observed at week 5 and remained at steady state to week 38. Animals terminated following scheduled dose administration also exhibited multifocal nerve degeneration of dorsal root ganglia, peripheral nerves and spinal cord at all doses. Glomerulonephropathy was exhibited in males and females; 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. The HNSTD is 0.05mg/kg (0.6mg/m²). Toxicokinetic 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 based on low numbers of animals and only 5-6 timepoints measured following dosing.

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Rodents were administered PS-341 as a single dose, weekly for 8 and twice weekly for 2w and 26w. The 9cycle 26w study is discussed in detail below. The LD₁₀/HNSTD for multiple-dose studies ranged from 1.2-1.8mg/m². Findings included hepatic necrosis, and lesions of the kidneys, thymus, lymph nodes, colon and urinary bladder.

When rodents were dosed for 9-three week cycles of twice weekly dosing for 2w followed by 1w rest, mortality was observed at doses $\geq 0.90\text{mg/m}^2$ in 11/60 rodents. Mortality was due primarily to hematopoietic, gastrointestinal and lymphoid system debilitation. Female decedents 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. Reduced motor activity was exhibited at 0.6mg/m^2 . Neurotoxicity was multifocal and included brain dilatation, and degeneration of dorsal root ganglia, peripheral nerves and spinal cord; neurotoxicity was limited to rodents administered $\geq 0.9\text{mg/m}^2$. Thrombocytopenia was observed at all dose levels and anemia was observed at 0.6 and 0.9mg/m^2 . Depressed thymus weights, accompanied by lymphocyte depletion, was exhibited at $\geq 0.9\text{mg/m}^2$. Chronic progressive nephropathy was generally observed at 26w and following recovery; males appeared to be more susceptible to kidney changes. Lymphoid atrophy and/or necrosis was exhibited in thymus, spleen and lymph nodes. Histopathological changes in cardiac tissue were comparable to control animals with few exceptions (slight increased incidence of perivascular necrosis, degeneration, hemorrhage and inflammation) at the MD and HD. Following recovery, myocardial and vascular inflammation, and cardiac necrosis was observed sporadically at all doses; the incidence of findings was not dose dependent. The severity of cardiac findings was not reported; therefore, the effect of PS-341 on rodent cardiotoxicity could not be clearly determined. The HNSTD is 0.10mg/kg (0.6mg/m^2). Following the 8-week recovery period, there appeared to be some indication of reversibility of findings observed during dosing. A dose-dependent increase was exhibited in AUC and C_{max} at weeks 1, 14 and 26 and over the duration of dosing from week 1 to week 14. However, non-linearity was observed with increasing dose. Toxicokinetic data should be considered general estimates with only 4 timepoints measured following dosing at weeks 1, 14 and 26. A dose-dependent decrease in the 20S proteasome chymotryptic:tryptic ratio from 30m to 48h following dosing at weeks 1, 14 and 26; partial recovery was observed by 72h postdose.

Toxicology Conclusions:

Previous studies support animal models, dose levels (HD of 1.2mg/m^2 for both species) and schedules of 9- and 38-three week studies conducted in rodents and monkeys, respectively. 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.

Significant cardiotoxicity was not observed in monkeys administered PS-341 for 13 cycles at 1.2mg/m^2 PS-341; this correlates with previous findings of cardiotoxicity and cardiovascular collapse at doses $\geq 2.4\text{mg/m}^2$ exhibited in a cardiotoxicity study (see preclinical toxicology overview). However, minimal incidence of cardiac changes were observed in rodents and monkeys; the incidence of these findings was not dose dependent. However, cardiac findings did not reverse in rodents and were observed following recovery; moreover, the severity of cardiac

findings was not reported. The effect of PS-341 on rodent cardiotoxicity could not be clearly determined. 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_{max} were also exhibited in both species. However, exposure in HD rodents was non-linear (approximately $\frac{1}{2}$ exposure at the MD and LD 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.

In monkeys and rodents there exists only a small dose margin between lethality at doses $\geq 0.9\text{mg}/\text{m}^2$ and the MTD or HNSTD of $0.6\text{mg}/\text{m}^2$ following administration of PS-341. It is interesting to note that the lethal dose and MTD are the same in both species.

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Histopathology Inventory for NDA # 21,602

Study	57285	57284		
Species	Rat	Monkey		
Adrenals	X*	X*		
Aorta	X	X		
Bone Marrow smear	X	X		
Bone (femur)	X ^b	X		
Brain	X*	X*		
Cecum	X	X		
Cervix				
Colon	X	X		
Duodenum	X	X		
Epididymis	X*	X*		
Esophagus	X	X		
Eye	X	X		
Fallopian tube				
Gall bladder		X		
Gross lesions	X	X		
Harderian gland	X			
Heart	X*	X*		
Ileum	X	X		
Injection site	X	X		
Jejunum	X	X		
Kidneys	X*	X*		
Lachrymal gland	X	X		
Larynx				
Liver	X*	X		
Lungs	X*	X*		
Lymph nodes, bronchial	X			
Lymph nodes, mandibular	X	X*		
Lymph nodes, mesenteric	X	X*		
Mammary Gland	X	X		
Nasal cavity	X	X		
Optic nerves	X	X		
Ovaries	X*	X*		
Pancreas	X	X		
Parathyroid	X*	X*		
Peripheral nerve ^a	X	X		
Pharynx				
Pituitary	X*	X*		
Prostate	X*	X*		
Rectum	X ^b	X		
Salivary gland	X*	X*		
Sciatic nerve	X	X		
Seminal vesicles	X*	X*		
Skeletal muscle	X	X		
Skin	X	X		
Spinal cord	X	X		
Spleen	X*	X*		
Sternum		X		
Stomach	X	X		
Testes	X*	X*		
Thymus	X*	X*		
Thyroid	X*	X*		
Tongue	X	X		
Trachea	X	X		
Urinary bladder	X	X		
Uterus	X*	X*		
Vagina	X	X		
Zymbal gland				
Lymph node, axillary		X*		
Lymph node, tracheobronch.		X*		

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X, histopathology performed

*, organ weight obtained

^a Peripheral nerves included peroneal, sural, tibial; sciatic listed separately (included trigeminal nerve for monkey study)

^b Histopathology not conducted

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V. GENETIC TOXICOLOGY:

Reviewer name: Shwu-Luan Lee, Ph.D.

Study title: _____ ASSAY
_____) WITH A _____ ASSAY

Key findings: MG-341 (PS-341) was negative (not mutagenic) under test condition.

Study no: 095AW74.501001

Volume #, and page #: Electronic Module 4, 4.2.3.3.1.1

Conducting laboratory and location: _____

Date of study initiation: 06/16/95

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Lot # 5; _____ (Provided by Sponsor)

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Methods:Strains/species/cell line: *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538.

Dose selection criteria:

Basis of dose selection: Dose selection was based on the result of the preliminary toxicity assay.Range finding studies: In the preliminary toxicity assay, ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, and 5000 µg/plate) of the test article were plated, with an overnight culture of TA100 in both the presence and absence of rat liver S9 activation. No precipitate was observed but toxicity was observed at ≥ 67 µg per plate. Based on the findings of the toxicity assay, the maximum dose plated in the initial _____ assay was 333 µg per plate.Test agent stability:

The stability of the negative and positive control articles and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

The karyotypic stability of the cell line was assured by using the working cell stocks which were not beyond passage 20. The cell lot used was tested using the _____ staining procedure and found to be free of mycoplasma contamination.

Metabolic activation system: Aroclor 1254-induced rat liver S9.

Controls:

Vehicle: Dimethyl sulfoxide (DMSO)Negative controls: Vehicle controlPositive controls: See table below:

Positive Controls			
Strain	S9 Activation	Positive Control	Concentration ($\mu\text{g}/\text{plate}$)
TA98	+	2-aminoanthracene	1.0
	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	1.0
	-	sodium azide	1.0
TA1535	+	2-aminoanthracene	1.0
	-	sodium azide	1.0
TA1537	+	2-aminoanthracene	1.0
	-	9-aminoacridine	75
TA1538	+	2-aminoanthracene	1.0
	-	2-nitrofluorene	1.0

Footnote:

S9 was characterized by the ability to metabolize 2-aminoanthracene and dimethylbenzanthracene.

Comments:**Exposure conditions:**

Incubation and sampling times: The plates were incubated for approximately 48 to 72 hours at $37\pm 2^\circ\text{C}$. Plates that were not counted immediately following the incubation period were stored at $4\pm 2^\circ\text{C}$.

Doses used in definitive study:

Experiment B1/B2: 1, 3.3, 10, 33, 100, 333 $\mu\text{g}/\text{plate}$.

Experiment B3: 1, 3.3, 10, 33, 100, 333, 1000 $\mu\text{g}/\text{plate}$.

Study design: _____ method

Analysis:

No. of replicates: Triplicate.

Counting method:

Revertant colonies for a given tester strain and activation condition were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity.

Criteria for positive results: For a test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

Strains TA1535, TA1537 and TA1538: Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than three times the mean vehicle control value.

Strains TA98 and TA100: Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than two times the mean vehicle control value.

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Summary of individual study findings:

Study validity: The study has followed the criteria required for a valid test, in terms of

- spontaneous revertant background frequency based on historical control data ("The mean revertants per plate were within the following ranges (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; TA1538, 5 - 35.")
- tester strain titers must be $\geq 0.3 \times 10^9$ cells per milliliter (ICH concentration limit). In the confirmatory assays (Experiment B3) the tester bacteria plated was 0.7 to 1.8×10^8 cells/ml. None of the tester strains have been seeded to meet the limit.
- positive control values (mean positive control value exhibits \geq three-fold increase over the mean vehicle control for each tester strain).
- toxicity (a minimum of three non-toxic dose levels will be required to evaluate assay data).
- However, the tester strain integrity data was not included in the submission (

Study outcome:

- Three mutagenicity assays were performed, which included: Experiment B1, the initial assay, Experiments B2, the repeated initial assay, and Experiment B3, the confirmatory assay.
- In Experiment B1, no positive responses were observed with any of the tester strains in the presence of S9 activation and with tester strains TA98, TA100, TA1535 and TA1537 in the absence of S9 activation. In the repeated initial assay, Experiment B2, no positive response was observed with tester strain TA1538 in the absence of S9 activation.
- In Experiment B3, no positive responses were observed with any of the tester strains in the presence and absence of S9 activation.
- No precipitate was observed but toxicity was generally observed at $\geq 33 \mu\text{g}$ to $\geq 100 \mu\text{g}$ per plate.
- The positive controls induced satisfactory mutagenic responses.
- The mean vehicle control (revertants per plate) values fell in the range of historical data for all tester strains in the presence or absence of S9 activation.
- Conclusion: Under the study conditions of the _____ Assay _____, MG-341 (PS-341) did not cause a positive response with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9.

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The summary of data of the _____ assay:

Test Article Id : MG-341
 Study Number : G95AW74.501001 Experiment No : B3

Average Revertants Per Plate ± Standard Deviation
 Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	12 ± 3	116 ± 3	7 ± 1	4 ± 3	7 ± 4
1.0	13 ± 2	119 ± 19	7 ± 2	6 ± 3	6 ± 4
3.3	11 ± 2	111 ± 15	7 ± 3	2 ± 1	4 ± 3
10	13 ± 2	102 ± 7	7 ± 3	5 ± 3	9 ± 1
33	8 ± 2	94 ± 8	4 ± 1	2 ± 2	8 ± 2
100	9 ± 1	24 ± 2	2 ± 1	2 ± 0	6 ± 3
333	2 ± 1	12 ± 4	2 ± 1	0 ± 1	3 ± 2
1000	0 ± 0	6 ± 7	0 ± 0	0 ± 0	1 ± 1
Pos	133 ± 9	353 ± 11	260 ± 2	932 ± 148	184 ± 35

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	22 ± 7	131 ± 4	7 ± 3	6 ± 1	8 ± 3
1.0	16 ± 2	125 ± 13	8 ± 1	7 ± 3	9 ± 3
3.3	20 ± 7	145 ± 15	9 ± 4	3 ± 3	8 ± 2
10	20 ± 1	149 ± 15	7 ± 2	3 ± 1	8 ± 3
33	18 ± 12	43 ± 2	6 ± 2	5 ± 2	7 ± 1
100	8 ± 1	16 ± 3	2 ± 1	3 ± 3	5 ± 1
333	5 ± 1	6 ± 4	1 ± 1	2 ± 1	1 ± 2
1000	3 ± 2	2 ± 2	0 ± 1	1 ± 1	0 ± 0
Pos	1000 ± 28	646 ± 453	70 ± 5	98 ± 12	867 ± 93

0.0 = Vehicle plating aliquot of 50 µl
 Pos = Positive Control concentrations as specified in Materials and Methods section.

Study title: _____ Assay with PS-341

Key findings: PS-341 was negative (non-mutagenic) in the _____ assay under the conditions tested.

Study no: AA45LR.503.BTL

Volume #, and page #: Electronic Module 4, 4.2.3.3.1.2

Conducting laboratory and location: _____

Date of study initiation: 02, May 2002

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: 010168; _____ provided by Sponsor)

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Methods:

Strains/species/cell line: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2 *uvrA*.

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Dose selection criteria:

Basis of dose selection: dose levels were selected following a preliminary toxicity test. Two Initial _____ Assays (B1 and B2) were conducted. In Experiment B1, dose levels of PS-341 tested were 0.33, 1.0, 3.3, 10, 33, 100, 333 and 1000 µg per plate (nominal). In Experiment B2, dose levels of PS-341 tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate (nominal). No precipitate was observed at all dose levels, but toxicity was observed beginning at 50 µg/plate (nominal) with some conditions.

Range finding studies:

Based on the findings of the initial toxicity-mutation assay, the maximum PS-341 doses plated in the initial _____ assay were 1500 µg per plate (nominal) with tester strains TA98, TA100, TA1535 and TA1537 in the presence of S9 and with tester strains TA100 and TA1535 in the absence of S9 activation and 5000 µg per plate (nominal) with the remaining conditions. The doses used in the _____ Assay were listed in "Doses used in definitive study".

Test agent stability: Stable for up to 6 months.

Metabolic activation system: Aroclor 1254-induced rat liver S9. The preparation of S9 has followed the conventional methods.

Controls:

Vehicle: Dimethyl sulfoxide (DMSO)

Negative controls: Vehicle control

Positive controls: the table below (from the sponsor) summarizes positive controls used in this study:

Strain	S9	Positive Control	Concentration (µg/plate)
<i>Salmonella</i> Strains	Rat	2-aminoanthracene	1.0
WP2 <i>uvrA</i>			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 <i>uvrA</i>		methyl methanesulfonate	1,000

Footnote:

S9 was characterized by the ability to metabolize 2-aminoanthracene and dimethylbenzanthracene.

Exposure conditions:

Incubation and sampling times: Plates with test strains and test articles were incubated for approximately 48 to 72 hours at 37±2°C. Plates that were not counted

immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

Doses used in definitive study:

See "summary of results" in "Study outcome", below.

Study design: Plate incorporation methodology

Analysis:

No. of replicates: All dose levels of test article, negative controls and positive controls were plated in triplicate.

Counting method: Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

Criteria for positive results: For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

Summary of individual study findings:

Study validity: Valid. The study has followed the criteria required for a valid test, in terms of:

- Tester strain integrity
- Spontaneous revertant background frequency : The mean revertants per plate were within the following ranges (inclusive): TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 *uvrA*, 10 - 60.
- Tester strain titers must be $\geq 0.3 \times 10^9$ cells per milliliter: In this study the tester bacteria plated was 1 to 5.6×10^8 cells/50 μ l, or 2 to 11.2×10^9 cells/ml.
- Positive control values: mean positive control value exhibits \geq three-fold increase over the mean vehicle control for each tester strain.
- Toxicity: a minimum of three non-toxic dose levels will be required to evaluate assay data. Toxicity was observed in plates containing PS-341 ≥ 50 μ g/plate. Thus the tests have included at least three no-toxic dose levels: 1.5, 5, and 15 μ g/plate.

Study outcome:

- Two Confirmatory Assays, B3 and B4, were performed. No positive mutagenic responses were observed with tester strains TA98, TA100, TA1537 and WP2 *uvrA* in the presence and absence of S9 activation.

- No precipitate was observed but toxicity was observed beginning at 50, 150 or 1500 µg per plate (nominal) with some conditions.
- Tester strain TA1535 in the presence and absence of S9 activation was retested in Experiment B4 due to tester strain contamination. Tester strain TA98 in the presence of S9 activation was also retested in Experiment B4 due to lack of toxicity. No positive mutagenic responses were observed with any of the testing conditions in B4. Neither precipitate nor toxicity was observed.
- The tables below summarize results of Experiment B3 and Experiments B4, respectively:
- Conclusion: PS-341 did not induce mutagenic effects in the bacterial reverse mutation assay under the conditions tested.

Bacterial Mutation Assay with PS-341
Summary of Results

Table 26

Test Article Id	: PS-341			
Study Number	: AA45LR.503.BTL		Experiment No : B3	
Average Revertants Per Plate ± Standard Deviation				
Liver Microsomes: None				
Dose (µg/plate)	TA98	TA100	TA1537	WP2 uvrA
Vehicle	12 ± 1	170 ± 6	11 ± 3	10 ± 0
1.5		175 ± 19		
5.0	15 ± 2	189 ± 12	10 ± 4	11 ± 1
15	13 ± 3	167 ± 18	5 ± 1	10 ± 1
50	14 ± 2	116 ± 11	8 ± 4	12 ± 2
150	10 ± 5	53 ± 4	5 ± 2	14 ± 0
500	13 ± 2	51 ± 16	4 ± 1	14 ± 2
1500	4 ± 1	45 ± 4	3 ± 1	8 ± 3
5000	4 ± 1		3 ± 1	8 ± 2
Positive	101 ± 18	582 ± 28	545 ± 191	77 ± 8
Liver Microsomes: Rat liver S9				
Dose (µg/plate)	TA98	TA100	TA1537	WP2 uvrA
Vehicle	17 ± 3	197 ± 15	10 ± 4	13 ± 4
1.5	18 ± 3	199 ± 7	6 ± 2	
5.0	20 ± 1	200 ± 21	8 ± 1	15 ± 1
15	19 ± 2	198 ± 19	7 ± 2	16 ± 1
50	17 ± 1	92 ± 2	8 ± 2	14 ± 3
150	17 ± 1	45 ± 10	7 ± 5	16 ± 2
500	11 ± 4	26 ± 4	3 ± 1	14 ± 3
1500	11 ± 4	28 ± 11	3 ± 1	7 ± 2
5000				8 ± 2
Positive	371 ± 36	858 ± 43	60 ± 18	208 ± 36

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

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Bacterial Mutation Assay with PS-341
Summary of Results

Table 27

Test Article Id : PS-341		Experiment No : B4	
Study Number : AA45LR.503.BTL			
Average Revertants Per Plate ± Standard Deviation			
Liver Microsomes: None			
Dose (µg/plate)	TA1535		
Vehicle	14 ± 2		
0.50	14 ± 2		
1.5	13 ± 1		
5.0	12 ± 3		
15	16 ± 1		
50	15 ± 1		
150	15 ± 1		
500	14 ± 1		
1500	14 ± 2		
5000	11 ± 1		
Positive	253 ± 28		
Liver Microsomes: Rat liver S9			
Dose (µg/plate)	TA98	TA1535 :	
Vehicle	16 ± 1	12 ± 2	2
0.50	14 ± 1	11 ± 2	2
1.5	13 ± 1	12 ± 0	
5.0	14 ± 1	12 ± 2	2
15	16 ± 2	12 ± 1	
50	15 ± 3	10 ± 1	
150	13 ± 1	11 ± 1	
500	14 ± 2	9 ± 1	
1500	12 ± 2	11 ± 1	
5000	13 ± 1	12 ± 2	2
Positive	452 ± 129	91 ± 11	

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Study title: IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST IN CHINESE HAMSTER OVARY CELLS

Key findings:

- PS-341 was positive for the induction of structural chromosome aberrations, with and without metabolic activation.
- PS-341 was negative for the induction of numerical chromosome aberrations in CHO cells.

Study no: AA45LR.331.BTL

Volume #, and page #: Module 4, 4.2.3.3.1.3

Conducting laboratory and location: _____

Date of study initiation: 07 June 2001

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: 970087; 96.23%, (provided by Sponsor)

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Methods:

Strains/species/cell line: Chinese hamster ovary (CHO-K₁) cells

Dose selection criteria:

Basis of dose selection: dose levels were selected following a preliminary toxicity test and were based on cell growth inhibition relative to the solvent control. The selection was also based on solubility of PS-341.

Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was observed at 2142 µg/mL in the non-activated 4 and 20 hour exposure groups, and at dose levels > 642.6 µg/mL in the S9 activated 4 hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 250 to 2142 µg/mL for the non-activated 4 and 20 hour exposure groups and for the S9 activated 4 hour exposure group.

Range finding studies: The selection of dose levels for analysis of chromosome aberrations in CHO cells was based upon mitotic inhibition (the lowest dose which induced at least 50% reduction in mitotic index, relative to the solvent control) and two lower doses. Due to excessive reduction in mitotic index, relative to the solvent control, only the lowest two doses of the non-activated 4 hour exposure group and the lowest dose of the S9 activated 4 hour exposure group could be analyzed for chromosome aberrations. The doses used in the chromosome aberration assay are shown in the table below:

Treatment Condition	Treatment Time	Recovery Time	PS-341 dose levels (µg/mL)
-S9	4 hr	16 hr	3.125, 6.25, 12.5, 25, 50, 100, 200
	20 hr	0 hr	1.56, 3.125, 6.25, 12.5, 25, 50, 100
+S9	4 hr	16 hr	3.125, 6.25, 12.5, 25, 50, 100, 200

Test agent stability:

The karyotypic stability of the cell line was assured by using the working cell stocks which were not beyond passage 20. The cell lot used was tested using the staining procedure and found to be free of mycoplasma contamination.

Metabolic activation system: Aroclor 1254-induced rat liver S9.

Controls:

Vehicle: Dimethyl sulfoxide (DMSO)

Negative controls: Solvent control

Positive controls: Mitomycin C (in the non-activated test system), Cyclophosphamide C (in the S9 activated test system).

Exposure conditions:

Incubation, sampling time, as well as doses used in definitive study are shown below:

Treatment Condition	Treatment Time	Recovery Time	PS-341 dose levels (µg/mL)
-S9	4 hr	16 hr	3.125, 6.25, 12.5, 25, 50, 100, 200
	20 hr	0 hr	1.56, 3.125, 6.25, 12.5, 25, 50, 100
+S9	4 hr	16 hr	3.125, 6.25, 12.5, 25, 50, 100, 200

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Study design:

Analysis:

No. of replicates: duplicated

Counting method: Twenty hours after initiation of treatment (approximately 1.5 normal cell cycles) the cells were harvested by trypsinization and counted using

_____ and the cell viability was assessed using _____ exclusion. Two hours prior to cell harvest, _____ was added to cell culture at a final concentration of 0.1 µg/ml.

Mitotic index: the percentage of cells in mitosis per 500 cells scored. Metaphase cells with 20±2 centromeres were examined.

% Aberrant cells: A minimum of 200 metaphase spreads were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask was reduced when the percentage of aberrant cells reached a statistically significant level before 100 cells were scored.

Criteria for positive results: The percentage of cells with chromosome aberrations in the flasks treated with test articles must be statistically increased ($p < 0.05$, Fisher's exact test) relative to the positive control.

Summary of individual study findings:

Study validity: Valid, since the study has followed the regulation in terms that:

- The frequency of cells with structural chromosome aberrations in the solvent control was within the range of the historical solvent control (0-6 %).
- The percentage of cells with chromosome aberrations in the positive control (12%) was statistically increased relative to the solvent control.

Study outcome:

- Toxicity of PS-341 (cell growth inhibition relative to the solvent control) in CHO cells treated for 4 hours in the absence of S9 activation was 41% at 12.5 µg/mL. The mitotic index at the highest dose level evaluated for chromosome aberrations, 12.5 µg/mL (4.8% and 5.6% for two flasks), was reduced by 19% relative to the solvent control.
- The dose levels selected for microscopic analysis were 3.125, 12.5, and 100 µg/mL. Due to the lack of scorable metaphase cells, the dose level of 100 µg/mL could not be scored for chromosome aberrations and fewer than 200 cells per dose were scored at dose levels of 3.125 and 12.5 µg/mL.
- The percentage of cells with structural aberrations in the PS-341 treated groups was significantly increased above that of the solvent control at dose levels 3.125 and 12.5 µg/mL (20.5% and 36%, respectively; $p < 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$).
- The percentage of cells with numerical aberrations in the PS-341 treated groups was not significantly increased above that of the solvent control, regardless of dose level.

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SUMMARY								
Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Structural (%)	
DMSO	-	4	6.4	200	0.000 ±0.000	1.5	0.0	
PS-341								
3.125	-	4	4.7	83	1.687 ±3.649	0.0	20.5**	
12.5	-	4	5.2	25	2.680 ±4.269	0.0	36.0**	
100	-	4	3.1	0				
MMC 0.2	-	4	1.9	200	0.125 ±0.346	1.5	12.0**	
DMSO	+	4	7.8	200	0.000 ±0.000	0.5	0.0	
PS-341								
3.125	+	4	4.6	46	1.913 ±3.811	0.0	23.9**	
6.25	+	4	5.1	0				
12.5	+	4	3.8	0				
CP 10	+	4	5.7	200	0.315 ±1.123	1.5	17.0**	

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
 Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
 Percent Aberrant Cells: *, p<0.05; **, p<0.01; using Fisher's exact test.

Study title: MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST IN MICE

Key findings: PS- 341 was not clastogenic in the mouse micronucleus assay under conditions of the assay.

Study no: AA45LR. 123. BTL

Volume #, and page #: Electronoc Module 4, 4.2.3.3.2

Conducting laboratory and location: _____

Date of study initiation: June 19, 2001

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot # and % purity: 258388; _____ (Provided by Sponsor)

Formulation: Each vial of PS- 341/ mannitol contained 3.5 mg of the active ingredient PS- 341 and 35 mg of mannitol. Each vial of was reconstituted with 3.5 mL of sodium chloride for injection, USP, resulting in 1 mg/ mL PS- 341 and 10 mg/ mL. The solution was used within two hours of reconstitution.

Vehicle: Test article vehicle (mannitol in 0.9% saline, USP)

Methods:

Species: ICR mice. Mice were approximately 6-8 weeks old. Body weights of the mice were within the range of 25-30 gm.