2. G465502A: Cardiotoxicity of PS-341 (NSC- D681239) in the Monkey (G465502A) Volume 4.2.1.3.2.

Key Study Findings: The data suggest that PS- 341 elicits a dose-dependent and reversible hypotensive response when administered IV (bolus) at doses up to 0.2 mg/ kg. Higher doses (0.25 and 0.30mg/kg) were lethal due to profound hypotension. No changes in ECG were noted at any dose level.

Conducting Laboratory and Location: [redacted]
Date of Study initiation: February 3, 1998
GLP Compliance: NO
Species and strain: Cynomolgus Monkeys
#/sex/group: 1/male/dose (n=4 total)
Weight: 4.6 – 4.9 kg
Drug, lot #: The test material, PS-341, Lot No. 5; [redacted], pure. It was received at [redacted] on February 3, 1998.
Formulation/vehicle: 2% Ethanol 0.9% Sodium Chloride for Injection, USP
Dose/Route/Volume/Duration: 0.1mg/kg (1.2mg/ m^2), 0.2 mg/kg (2.4 mg/m^2), 0.25 mg/kg (3.0 mg/m^2) and 0.3 mg/kg (3.6 mg/m^2) PS-341, IV Bolus in 0.2, 0.4, 0.5, and 0.6 mL/kg
Observations:

Clinical Observations: Animals observed at approximately 1, 3, 6, and 12 hours post-dosing and at least twice daily during the remainder of the study, or more often as clinical signs warranted.

Body Weights: Body weights were recorded prior to study start.

Heart Rates, Blood Pressures, Body Temperatures and ECGs: Heart rates, blood pressures, body temperatures and ECGs were recorded from all animals via implanted cardiovascular radiotelemetry devices. Data were collected for a minimum of 24 hours prior to test article administration. Data were collected from animals until either eight days following dosing or until the animal was euthanized.

Clinical Pathology: Blood for clinical pathology evaluation was collected prestudy to aid in the selection of animals for inclusion on study.

Scheduled Necropsy- no scheduled necropsy, animals to be returned to batelle stock.

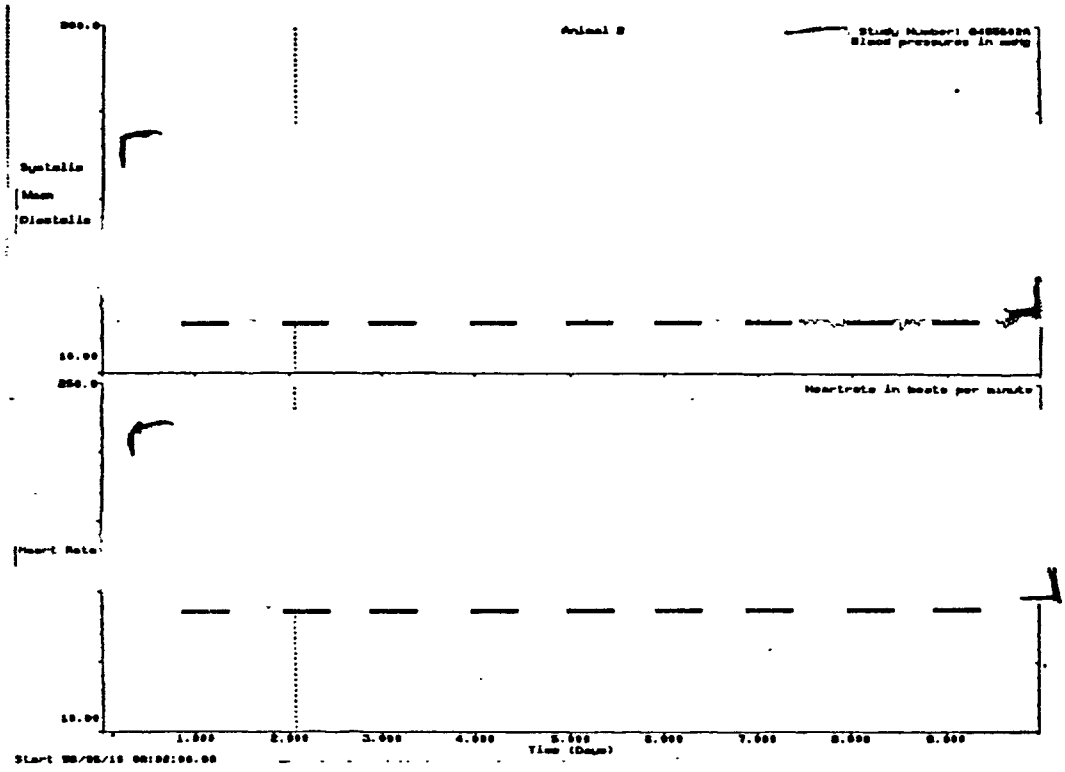
Dose Levels, Clinical Observations and Cardiovascular effects:

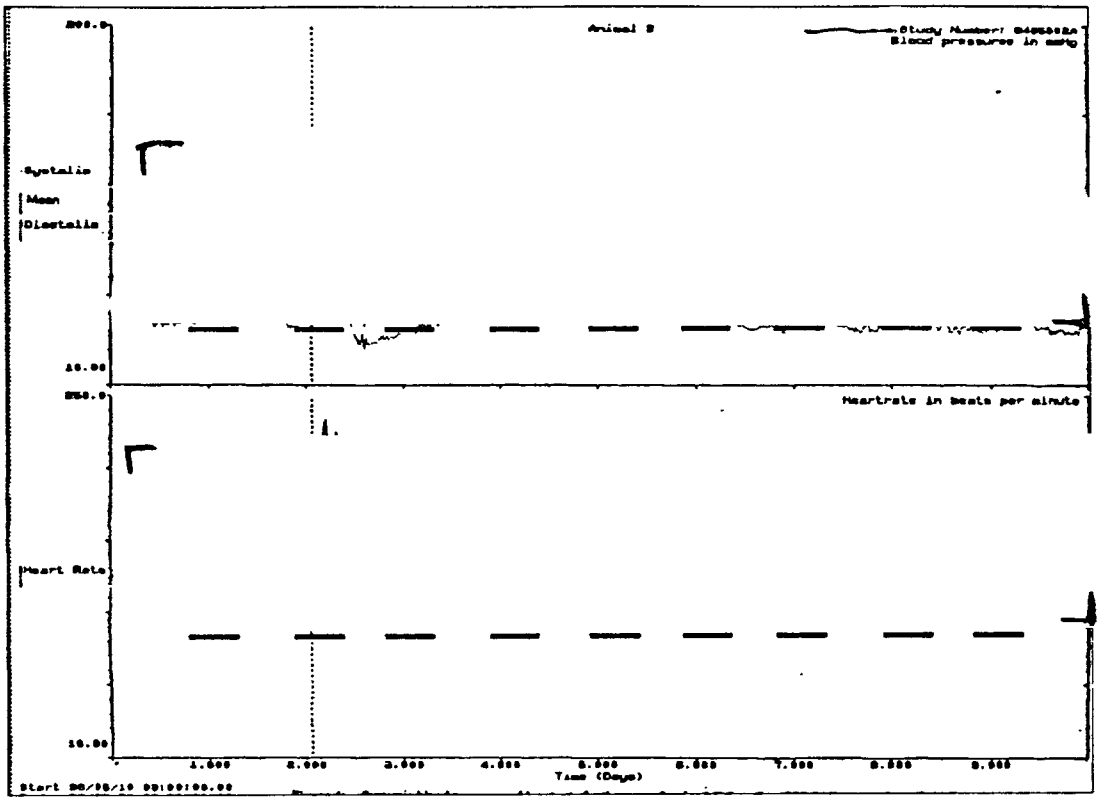
Dose Levels			Clinical Observations	Cardiovascular Effects
mg/kg	mg/m^2	AUC_{1-24} (hr*ng/ml)		
0.1	1.2	51.3 ± 10.6[A]	No unusual signs	↑ Heart rate (0 -24 hours post dose)*
0.2	2.4	None	Emesis	↑↑ heart rate; ↑ body temp.; ↓BP (all reversible by 96 h)
0.25/ 0.30	3.0/ 3.6	None	Emesis, diarrhea, lethargy. **Moribund 13- 14 hrs post dose (Resulting in sacrifice)**	↑↑ heart rate; ↑ body temp.; ↓↓↓ BP

[A]From study rpt-RPT- 00039: Toxicokinetic Study Report Supporting a 38- week (13 cycles) Intravenous Injection Toxicity Study of PS- 341 in the Cynomolgus Monkey

Below are traces of telemetry data gathered on blood pressure and heart rate to reflect the timing, magnitude and duration of changes.

Systemic blood pressure and heart rate for one animal/dose that received the low dose (0.1 mg/kg of PS-341)(top), the moderate dose (0.2 mg/kg of PS-34 I) (middle), and high dose (0.25 mg/kg) pretest through 10 days post dosing. Displays heart rate in beats per minute and blood pressure in mmHg (measured every 30 minutes). The vertical dashed line represents the time at which the animal was dosed (which occurred between 9AM and 10 AM). Horizontal dashes represent approximate times at which lights are on.





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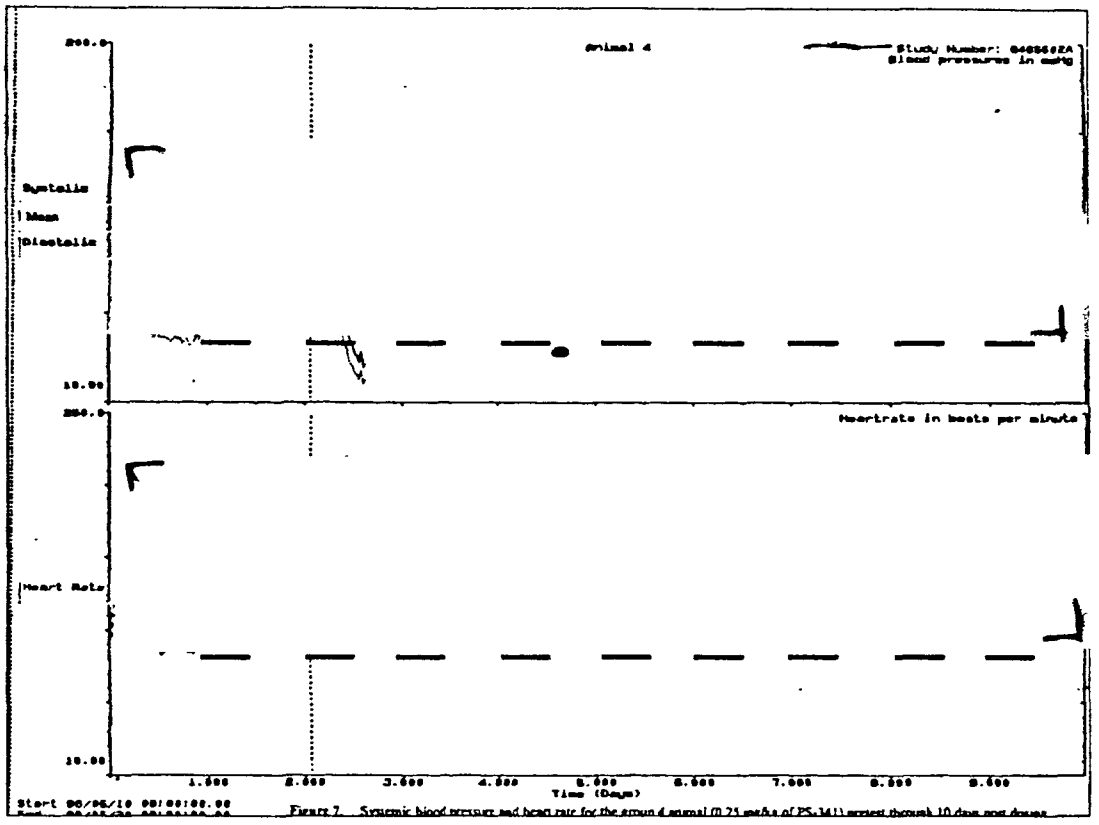


Figure 7. Systemic blood pressure and heart rate for the group 4 animal (0.25 mg/kg of PS-341) arrested through 10 dose post dosage

At doses of 0.25 and 0.3 mg/kg, physiologically significant heart rate elevations generally preceded a profound hypotension.

> **Comment:** The heart rate increase was possibly a compensatory response to the hypotension caused by decreased peripheral resistance.

Portions of the review of this study are excerpted from review of IND No ——— (Oct. 13, 1998; Hua Zheng, Ph.D)

3. KLAW- 191: A study to determine the effects of PS- 341 on cardiovascular function after intravenous administration to anesthetized cynomolgus monkeys. Volume 4.2.1.3.3.

Conducting Laboratory and Location: [redacted]

Date of Initiation: May 8, 2002
GLP Compliance: NO
Species and strain: Cynomolgus Monkeys
#/sex/group: see table for study design
Dose/Route/Volume/Duration: see table for study design

Study Design

Group Number	Number of Animals: Males	Number of Animals: Females	Test Article[a]	Dosage Level (mg/kg)	Dosage Level (mg/m^2)	Dosage Volume (mL/kg)[b]	Dosing Regimen	Observation Period
1	1	1	PS-341	0.03	0.36	0.06	SD (IV)	Up to 6 hours post dose
2	1	1	PS-341	0.3	3.6	0.6	SD (IV)	
3	1	1	PS-341	0.5	6	1.0	SD (IV)	
4[c]	1	1	[redacted]					

IV = intravenous; SD = single dose
a: All animals received the D- mannitol control dose at the same concentration as the mannitol in the test article concentration for each appropriate study day, ~30 minutes prior to the test article administration.
b: Volume did not exceed 10 mL/ kg.
C: Study was terminated prior to dosing of Group 4.

Weight: 4.1 to 4.5 kg (males); 3.2 to 3.6 kg (females).
Drug, lot #, purity: The test material, PS-341, Lot No. D2-1-1, is a white powder and is [redacted] pure. It was received at [redacted] on May 2, 2002. A re-test date of July 17th, 2002 was provided.
Formulation/vehicle: D- mannitol in saline
Observations:

1. Heart rate (HR), direct blood pressure (systolic [SAP], diastolic [DAP], and mean arterial pressure [MAP], derived), central venous pressure (CVP), left ventricular pressure (LVP) (systolic and diastolic), and contractility (dP/ dt) parameters were monitored continuously. Cardiac output (CO) and BT were recorded approximately every 30 minutes. A catheter was placed in a femoral or jugular vein and advanced into the caudal vena cava for measurement of CVP, and collection of blood. A [redacted] catheter was placed in a femoral or jugular vein and advanced into a pulmonary artery for measurement of CO, PAP, and BT. A catheter was placed in a femoral artery and advanced into the aorta for measurement of SAP, DAP, and MAP. Another catheter was placed in a femoral or carotid artery and advanced into the left ventricle for measurement of LVP and calculation of dP/ dt (max).

   Values were collected as 10- second waveform scans. Parameters were saved and were reported according to the schedule in the following table:

Time Duration	Reported (Approximate)
Baseline = ~15- 30 min prior to treatment	Baseline mean
Vehicle treatment to 30 min post treatment	Every 5 minutes (averages)
Test Article treatment to 6 hrs post treatment	Every 5 minutes (averages)

2. Electrocardiogram (ECG) tracings were obtained using a [redacted] or equivalent approximately every 30 minutes, at 50 mm/ sec for 10 seconds using limb leads I, II, and III, and augmented leads aVR, aVL, and aVF. Three leads were monitored simultaneously.

   Baseline ECG tracings were analyzed as well as 30 minutes to 6 hours following test article treatment, every 30 minutes.

3. Blood samples were collected at baseline, 1 hours post treatment and at the end of the study.

Mortality: *none was observed; however animals were sacrificed at the end of the 6 hour observation period.*

Comment: Detection of moribund animals in previous studies occurs >12 hours following dosing therefore, current study design appears inadequate to address issues of drug associated mortality as observed in previous studies.

Cardiovascular Monitoring:

Observed Adverse ECG Events:

SEX	Dose	Timeframe	Abnormality
Male (1001)	0.03 mg/kg	Pre-dose	Ventricular Premature Complex
		4 Hours	Ventricular Premature Complex
Male (2001)	0.30 mg/kg	4 Hours	Ventricular Premature Complex

No other events were noted in the cardiologist report.

Summary of Cardiovascular parameters evaluated in this study:

	Dose (mg/kg)		0.03 (0.36 mg/m²)		0.30 (3.6 mg/m²)		0.50 (6.0 mg/m²)	
	Sex		M	F	M	F	M	F
CO	% ▲ 6 H		↑43 %*	↑13%	↑28%	↑69%	0	↓43%
	Max ▲	%	↑51%	↑41%	↑55%	↑80%	↑36%	↑28%
		Time	5.5	5.0	4.5	4.5	2.0	0.5
HR	Max ▲	%	↑10%	↓10%	> ↑40%	~↑10%	~↑10%	↑40%
		Time	0.5 – 6	Steady from 1 H	4.0-6.0	1 –1.5 4.5 – 6	0.5 – 6.0	Steady ↑ from 0 - 3.5
MAP	% ▲ 6 H		↑10%	↓≤10%	↓ 20%	↓ 40%	↓ 30%	↓ ~100%
	Max ▲	%	±20%	±10%	↓ 0-25%	↓ 0-55%	± 35%	↑40-↓100%
		Time	↑ to 6 H; periodic fluctuations	↓ until 2.5H; steady at no change until 5.5H	Fluctuating but relatively **steady decline**	Fluctuating.	Fluctuating; spike (20 %) 1 H;Peak (35 %) 2 H, **steady decline**	Increasing until 1 H, **steady decline** thereafter
DP/dt (Contractility)			~ ↑50 % 1 – 6 H	± 50 % Flux, spike at 4.5	≥ ↑50% 0.5 to 6 H;(Max 300%) at 200% at 6H	≥ ↑50% 1 – 2H; again at 3- 5 H. Peak at 5H 300%	≥ 50% @ 1.25H-6 H. Up to 300% at 6H	↑ to 400% till 2.5; ↓ to 0% thereafter
SLVP			Unremarkable	± 50 %	Unremarkable	≤ ↓50%	↑ ing to 100% over 6 H	↑ to 50% till 4.5 H, ↓ to 0 %thereafter
DLVP (expressed as changes from baseline, relative to ambient)			Unremarkable	↑ 50 - 100 % 0-3 H; ↓ 250% 4.5H	↓ ≤ 50% to 3.5H	≤ ↑50%	↑ing 150%; ↓ to 225 % thereafter	↓ing to 100% till 6H
pMAP			Unremarkable	Unremarkable	↓≤50% 3.5 to 6H	Highly variable ±125%	± 50% duration	≤↑50% duration
CVP			Unremarkable	↓almost 100% at 1.5 – 6H*	↓ ≤ 75% 3.5 to 6 H	Highly varialble +200% to – 50%	↑ing 50% at2 H; ↓ to ≤ 100% after	≤↓ 50% until 6 H, where ↓ 100% is noted

CO- Cardiac Output, DP/dt- contractility, CVP- Central Venous Pressure, PMAP- Pulmonary Mean Arterial Pressure, DLVP- diastolic left ventricular pressure, SLVP- systolic left ventricular pressure

*Expressed * Potentially due to a shift in catheter, originally an extremely high number.

Cardiac Output: "relatively unchanged"-per sponsor

There is a general trend toward increasing cardiac output following administration independent of dose in all but one animal (female receiving 0.50mg/kg), when compared to baseline. Percent change (as compared to baseline) at six hours ranged from ↓43%-↑69%. There is no data to indicate that this is the result of decreased cardiac filling.

Heart Rate: *Following treatment with vehicle, heart rates were not effected in any of the animals from any of the treatment groups* (per sponsor). Individual changes listed above. Although with only two animals per group, it appears that there is a pattern of increases in HR following 0.3 and 0.5 mg/kg. Increases of >10% were evident at approximately 1 hour and endured for up to six hours.

Mean Arterial Pressure: Although quite variable, the intensity of the effect on arterial blood pressure seemed to increase in a dose-dependent manner following treatment with PS-341. With only two animals per treatment group, variable responses within the same treatment group, and with the complication of evaluating the contribution of anesthesia on changes in arterial blood pressure, statistically significant effect of PS- 341 on arterial blood pressure cannot be concluded, however these results point toward dose dependant decreases on MAP. See graphs below.

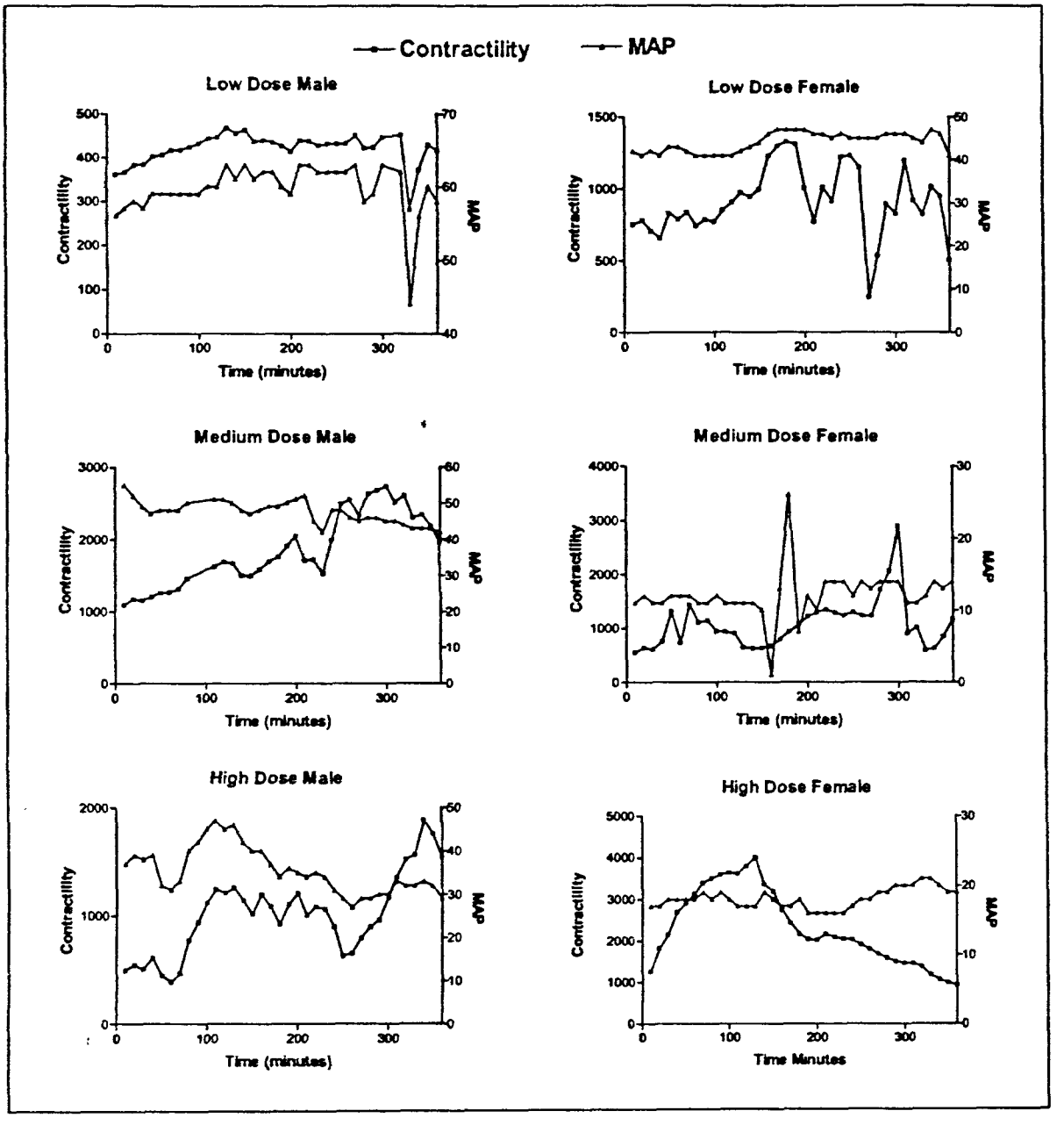
Left Ventricular Pressure: Following treatment with 0.03 mg/ kg PS- 341, left ventricular systolic pressure (sLVP) gradually increased over the six hours of monitoring to a level of increase that was approximately 20%- 40% greater than the baseline values Left ventricular diastolic pressure (dLVP) changed ± 4 mmHg over the monitoring period. Animals at 0.30 mg/ kg PS- 341 demonstrated a similar level of effect on sLVP and dLVP as was observed at 0.03 mg/ kg PS- 341. Animals treated with 0.50 mg/ kg PS- 341 experienced a 60- 90% increase in sLVP, while dLVP generally decreased to 0 mmHg during the period of post- dose monitoring.

Ventricular Contractility: Maximal left ventricular contractility fluctuated about ± 40% over the six- hour monitoring period in the Group 1 treated animals. In animals receiving 0.30 mg/kg PS 341, dP/ dt increased at a gradual rate during the monitoring period to a peak level of approximately 300% at five hours after dosing. Both animals demonstrated increased dP/ dt values following PS- 341 treatment (300 – 400%), although the patterns of increase were different between the animals. Animal No. 3001 peaked at 150% above baseline values approximately two hours post dose. Values decreased to 50% above the baseline value at four hours post dose and subsequently increased over the last two hours of monitoring to approximately 300%. The female receiving 0.50mg/kg also experienced a single peak increases in dP/ dt in the same 300% range at two hours, and then experienced a gradual decline. The company asserts that "*Although the initial baseline values for dP/ dt were low, a dose- related response toward increasing values following treatment with PS- 341 was observed, suggesting a positive ionotropic effect*".

Comment: Toxicokinetic analysis of plasma concentrations does not support direct receptor agonism or antagonism, however, there is data to suggest that PS341 is sequestered in cardiac tissue, in comparison to other musculature.

PMAP: Although the percent change from baseline values often seemed large, the values in mmHg were within physiologic normal ranges.

CVP: Animals at 0.30 and 0.50 mg/ kg PS- 341, respectively demonstrated ± 50% changes in CVP during the six hours of monitoring following treatment. Although changes in CVP were observed, a consistent dose- related effect following treatment with PS- 341 on CVP was not observed during the period of monitoring.).



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Body Temperature: BT varied between a low of 32.8 C and a high of 38.1 C, with most animals not changing more than 4 C during the period of monitoring (there is no dose dependent pattern to changes). However, animals were maintained on a water jacket to maintain BT, therefore although the data do not indicate a dose dependant change in BT this may have been masked by heating.

Toxicokinetics and Proteasome Activity: A dose- dependent decrease in 20S proteasome specific activities was observed between 0.03 and 0.30 mg/ kg (see below). A similar dose- dependent decrease was not observed between 0.30 and 0.50 mg/ kg due to the near maximum reduction in proteasome activity obtained at 0.30 mg/ kg. No gender difference in response to PS- 341 was observed. At all dosages, 20S specific activity showed some recovery by six hours post dose as compared to one hour post dose values. Of note, the time-course PS-341 plasma distribution does not adequately mirror the timecourse of tissue distribution, and the actual activity may vary considerably by tissue. (See PK review by Anwar Goheer). Levels in the heart (0.359 and 0.167 µg eq/g at 1 hour and 6 hours post-dosing respectively) were greater than other muscular tissue, in comparison to tissue types other than those involved in excretion [(Adrenal gland 0.708(1 hour), 0.254 (6 hour); Pituitary gland 0.498 (1 hour) and 0.231 (6 hour)] concentrations.

	Dose (mg/kg)	0.03		0.30		0.50	
	Sex	M	F	M	F	M	F
Plasma [PS341] (ng/mL)	1 Hour	1.43	0.65	20.80	71.80	43.40	40.10
	6 Hour	LLQ	LLQ	2.00	9.93	5.46	4.97
Proteasome Specific Activity in Whole Blood (% Pre-dose activity)	1Hour	40	38	12	11	6	8
	6 Hour	53	56	22	17	13	17

4. APPENDIX A: RPT-00221-INVESTIGATIVE CARDIOVASCULAR STUDY OF THE PROTEOSOME INHIBITOR PS-341 IN THE MOUSE

Key Study Findings:

- Mice receiving 3 and 10 mg/kg (30mg/m2) experienced transient tachycardia (10 % ↑ until 30 minutes post-dose) followed by prolonged bradycardia (> 50% maximal decrease; beginning 30 minutes post dose continuing decline to the end of observation) in animals receiving 10 mg/kg
- Analysis of serum biochemistry indicated that animals receiving 10mg/kg were hypokalemic (↓40%)
- ALT and AGT- > 100% ↑ following 3mg/kg and ≥ 400% ↑ following 10 mg/kg
- LDH ≥ 100% ↑ following 10mg/kg
- Changes in HR following 10mg/kg was abrogated by maintaining body temp.
- Atropine partially attenuated decreases in HR following 10 mg/kg
- Ex vivo assessment of myocardial mechanical parameters determined that there were no differences in heart rate, left ventricular pressure, rates of pressure develpoment (dP/dt max), and decay (dP/dt min) and time constant of isovolumic relaxation (tau) 4 hour-postdose following a single IV bolus of PS-341 at 10 mg/kg when compared to saline.

Conducting Laboratory and Location: Millenium Pharmaceutics
Date of Study initiation: not provided
GLP Compliance: NO
Species and strain: BALB/c Mice
#/sex/group: 3-5/ female/group
Weight: 18 – 22 grams
Drug, lot #: The test material, PS-341, Lot No. not provided
Formulation/vehicle: saline
Dose/Route/Volume/Duration:

Experiment 1: IV/saline, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg (0.3, 0.9, 3.0, 9.0, and 30.0 mg/m^2, respectively) (n=5)

Observations:

ECG:Gel coated ECG electrodes were embedded in the floor of the platform. Electrodes were connected to an amplifier by three shielded conductive leads. Baseline data collected after mouse was acclimatized for 10 minutes. Signals were digitized with 16 bit precision at a sampling rate of 2000 samples/second. Parameters collected every 30 minutes up to 6.5 hours post-dose.

Serum: Collected 6.5 hours postdose at time of euthanasia via cardia puncture to determine if bradycardia was associated with electrolyte changes.

Scheduled Necropsy- 6.5 hours post-dose

Experiment 2: IV/ saline or 10 mg/kg; with and without temperature control to determine if decreasing body temperature altered HR (n=10)

Observations:

ECG: recordings taken at 0, 2, 4, and 5 hours post-dose.

Surface Body Temperature: Recordings taken at 0, 2, 4, and 5 hours post-dose via infrared thermometer

Experiment 3: IV/ saline, 10mg/kg PS341, or 10 mg/kg PS-341 + 0.5mg/kg Atropine to ascertain if bradycardia associated with PS341 administration is due to increased vagal tone (n=3)

Observations:

ECG: recordings taken at 0, 2, 4, and 5 hours post-dose

EXPERIMENT 4: IV/ SALINE OR 10MG/KG PS341

Hearts were harvested 4 hours post-dose and subjected to ex vivo Langendorff perfusion to monitor cardiac contractile parameters. (Previous studies indicate that PS341 distributes to heart tissue)

Observations:

Utilizing an IV balloon-catheter system specially designed for the mouse heart. Signals were digitized an stored every 0.5 milliseconds. Measurements included Left ventricular pressure, peak systolic pressure, end-diastolic pressure, and peak rates of pressure development and decline.

Results:

(Individual (raw or summarized) data were not provided. The results are based on the sponsors interpretation and graphs provided by the sponsor and are therefore approximations)

ECG results are not presented for each experiment, but are presented as a general result. It is reported that ECG monitoring occurred in experiments 1, 2, and 3, the results indicate that

ECG tracings indicate a prolonged PR and QRS interval following 10 mg/kg (30 mg/m2) beginning ~30 minutes post-dose, continually increasing until end of observation.

Experiment 1(euthanized 6.5 hours post-dose)

- Mice receiving 3 (9 mg/m2) and 10 mg/kg (30mg/m2) experienced transient tachycardia (10 % ↑ until 30 minutes post-dose) followed by prolonged bradycardia (> 50% decrease; beginning 30 minutes post dose continuing decline to the end of observation) in animals receiving 10 mg/kg
- Analysis of serum biochemistry indicated that animals receiving 10mg/kg were hypokalemic (↓40%)
- ALT and AGT- > 100% ↑ following 3mg/kg
  ≥ 400% ↑ following 10 mg/kg
- LDH ≥ 100% ↑ following 10mg/kg

Results:

Group 1

Concentration of radioactivity (µg equiv/g) in blood and plasma at specified times postdose.

Group	Time (hour)	Blood	Plasma
1	1	0.15	0.02
	24	0.09	0.005
	48	0.08	0.005
	72	0.09	0.007

All samples reached their highest concentration at 1 hour postdose.

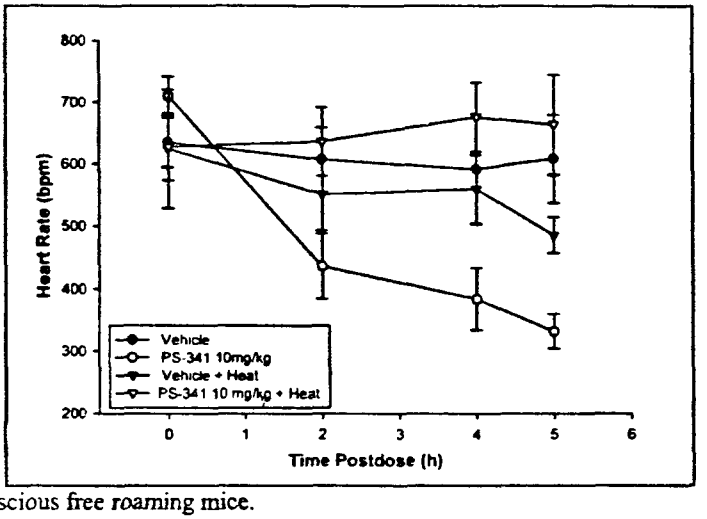
Mean concentration of radioactivity in blood and tissues at specified times postdose determined by tissue excision in male Sprague Dawley rats (Group 1) following single intravenous administration (0.25 mg/kg) of 14C-PS-341 are shown below.

		Radioactivity Concentration (µg equiv of ^{14}C-PS-341/g)			
		Time Point			
Tissue Type	Tissue	1 hour	24 hour	48 hour	72 hour
Vascular/	Blood	0.151	0 092	0 084	0 094
Lymphatic	Plasma	0.018	0.005	0 005	0 007
	Lymph nodes	0.396	0 452	0 463	0 485
Excretory/	Kidney	0.816	0 355	0.291	0.274
Metabolic	Liver	0.209	0 131	0 107	0 126
Gonads	Testis	0 023	0 024	0 024	0 027
Muscular	Myocardium	0 315	0 148	0 106	0 101
Unclassified	Bone marrow	0 465	0 284	0.218	0 182
	Lung	0 533	0 300	0 252	0 243
Alimentary	Stomach	0.339	0 138	0 109	0.107
Canal	Small intestine	0.184	0 056	0 033	0 032
	Upper GI-tract contents	0 465	0.035	0 011	0.008
	Cecum	0 350	0.215	0 150	0.139
	Large intestine	0 302	0.201	0 154	0 137
	Lower GI-tract contents	0 027	0 109	0 022	0 035
Ocular	Eye	0 042	0 026	0 022	0 020

The limit of quantitation was [redacted]

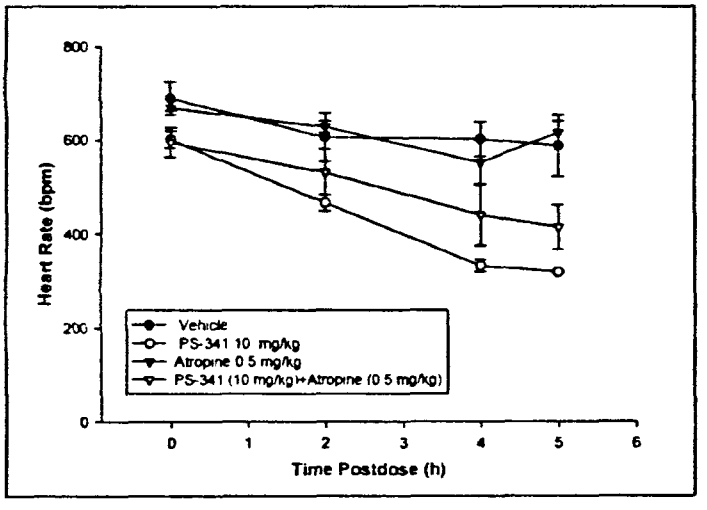
The highest concentrations of radioactivity were mainly in the organs of excretion and metabolism (kidney and liver). Bone marrow and lung also had a substantial concentration of radioactivity. Myocardium contained 0.32 and 0.1 µg equivalent ^{14}C-PS-341 at 1 and 72 hours postdose, respectively. Radioactivity concentration in CNS (cerebellum, cerebrum, medulla, spinal cord, pineal body, choroid plexus, and cerebrospinal fluid) was below the limit of quantification [redacted].

Experiment 2: Following the adminsitration of 10 mg/kg, there was a > 20 % decrease in body temperature. Supplemental heat attenuated the increase in HR associated with PS341 administration in



conscious free roaming mice.

Experiment 3: The effect of atropine and PS341 administration on heart rate in conscious free roaming mice. The sponsor concluded that atropine had no effect on PS341 in the presence or absence of Atropine. Graphic representation of the data appears to indicate that there is a trend toward partial attenuation of decreases in HR, but without individual data, statistical evaluation is not possible.



Experiment 4: The effects of PS-341 (10mg/kg) in the assay are presented in the table below.

Parameters	Saline Control (n=3)	PS-341 10.0 mg/kg (n=3)
LVP (mmHg)	61±16	82±28
EDP (mmHg)	8±3	10±2
dP/dt_{max} (mmHg/s)	2028±479	2581±880
dP/dt_{min} (mmHg/s)	1598±511	1739±449
Tau (ms)	16±2	16±2.3

Values are mean ± standard deviation (SD).

The sponsor concluded from these data that there were no direct effects of PS-341 on heart rate, left ventricular pressure, rates of pressure develpoment (dP/dt max), and decay (dP/dt min) and time constant of isovolumic relaxation (tau) 4 hour-postdose following a single IV bolus of PS-341 at 10 mg/kg when compared to saline. However, although there are not statistically significant changes there is a trend toward increases in all of these parameters.

Results:

- Mice receiving 3 and 10 mg/kg (30mg/m2) experienced transient tachycardia (10 % ↑ until 30 minutes post-dose) followed by prolonged bradycardia (> 50% maximal decrease; beginning 30 minutes post dose continuing decline to the end of observation) in animals receiving 10 mg/kg
- Analysis of serum biochemistry indicated that animals receiving 10mg/kg were hypokalemic (↓40%)
- ALT and AGT- > 100% ↑ following 3mg/kg and ≥ 400% ↑ following 10 mg/kg
- LDH ≥ 100% ↑ following 10mg/kg
- Changes in HR following 10mg/kg was abrogated by maintaining body temp.
- Atropine partially attenuated decreases in HR following 10 mg/kg
- Ex vivo assessment of myocardial mechanical parameters determined that there were no differences in heart rate, left ventricular pressure, rates of pressure develpoment (dP/dt max), and decay (dP/dt min) and time constant of isovolumic relaxation (tau) 4 hour-postdose following a single IV bolus of PS-341 at 10 mg/kg when compared to saline.

Conclusions:

Although these studies indicate significant cardiovascular anomalies (↑HR, followed by prolonged bradycardia) following PS341 administration, the studies are inadequate to elucidate the physiological basis of the hypotension in monkeys for numerous reasons.

- Extensive ——— studies have not been conducted in mice. Given that significantly higher doses are tolerated in mice (up to 30 mg/m2) than compared to rats and monkeys, it is uncertain if the exposure profile is similar between species.
- Doses utilized in this study were not shown to be lethal doses in mice.
- The pattern of cardiovascular changes following administration is not parallel between the monkey and this mouse study. In this study HR increased up to 30 minutes following injection and then began a continuous prolonged decline, resulting in bradycardia, not tachycardia as seen in the monkey.

It is clear that maintaining body temperature in mice abrogated observed bradycardia and that atropine only partially remediated the change in HR following high doses of PS341. However given the non-parallel nature of changes of mice and monkeys, these studies provide very little evidence to the etiology of the effects of PS243 on cardiovascular parameters.

5. THE PROTEASOME INHIBITOR PS-341 INDUCES COX-2 IN MURINE AND HUMAN ENDOTHELIAL CELLS
Submitted as a poster presented by Drug Safety and Disposition, Millenium Pharmaceuticals

Methods: *Clear methodology is not included in the sponsors submission*

- [redacted]

Observations:

- PS-341 induced PGF1alpha expression (24 fold induction) and PGE2 expression (37 fold induction) when compared to control via RIA, without concomitant induction of nitric oxide. This is in contrast to induction with lactacystin which resulted in a 7 and 10 fold induction of PGF1alpha and PGE2 respectively. This is in contrast to thromboxane B2 and leukotriene B4 which were minimally affected (less than 3 fold change) by lactacystin and PS341.
- 10 μM PS-341 induced Cox-2 mRNA expression in:
  - mouse endothelial cells (in vitro) in a time dependant manner- mRNA appeared elevated at 2 hours when compared to one hour and remained elevated until 30 hours at which time levels decrease be remain detectable until 50 hours (the last time point).
  - Human endothelial cells
  - PBMCs of Cynomolgus monkey in vivo. Although this is indicated as in vivo, there is no description of the treatment of the animals.
- PS-341 induces both Hsp70 and Cox2 expression in human endothelial cells in vitro. The time course of induction in bEND3 cells began at 6 hours following injury and continued until 50 hours.
- PS-341 induced both Cox2 and Hsp70 protein expression in bEND3 cells when [redacted] evaluated.

Conclusions:

These data may suggest that Cox-2 and prostanoid-regulated vasodilation may mediate the observed hypotension in experimental animals. However, in vivo analyses of prostanoid levels have not been conducted and would add credence to the claim that prostanoids are mediating the hypotension and lethality observed following high doses in animals. Toxicokinetic analysis of monkeys receiving 0.1mg/kg (1.2 mg/m2) indicated that Cmax and $AUC_{0\text{-}24}$ were 81 ng/ml and 51 hr*ng/ml, respectively (as indicated by the TK review summary by Anwar Goheer). This corresponds to molarity of 210 nM and 132 hr*nM. This is 5 and 7 fold lower than the concentrations utilized in these assays, making the interpretation of these results questionable.

Overall Summary of Safety Pharmacology:

The results of multiple cardiovascular safety studies indicate that PS-341causes: (1) a dose dependant decrease in MAP (mean arterial pressure), (2) a 10-40% increase in HR, and (3) increased CO (cardiac output) following the acute administration of PS341 at doses between 0.3 mg/kg and 0.5 mg/kg doses. In these same studies, there was a dose dependant increase in ventricular contractility following administration of 0.03 mg/kg-0.5 mg/kg. These data suggest that there is a significant potential for adverse cardiovascular events following the administration of PS-341 at doses of 0.25 mg/kg or greater (3.0 mg/m^2 and above).

The company asserts in their discussion of study 6837-113 that, "*There were no remarkable changes in mean blood pressure, heart rate, or electrocardiographic measurements after administration of 0.2 or 0.3 mg/kg.*" In contrast, the company argues in the discussion of the KLAW191 study that while "*the initial baseline values for dP/ dt were low, a dose-related response toward increasing values following treatment with PS- 341 was observed, suggesting a positive ionotropic effect*" on the heart. In the latter study, changes in contractility were shown to persist at 6 hours following drug administration, whereas plasma concentration of PS-341 at this time point were only 10% of that seen at 1 hour post-administration. This suggests that the pharmacodynamic effects of PS-341 may not be directly related to plasma concentration

but may instead be dependent upon intracellular or 'target bound' kinetics. Moreover, there is data to suggest that PS-341 is sequestered in the myocardium (see Pharmacokinetics/Toxicokinetics section of this review; by Anwar Goheer). Thus, the prolonged effect of PS-341 on contractility, heart rate and blood pressure, and evidence of cardiac necrosis seen following repeated dosing at 0.1 mg/kg (see Toxicology section of this review; by Margaret Brower) may be dependent on or explained by the local disposition of the drug.

As indicated in the consult review by Anthony Proakis, Ph.D (Div. of Cardiorenal Drug Products; appended) in vitro heart or isolated myocardial preparations are generally accepted test systems used to determine direct inotropic (positive) effects of drugs.[1] Ex vivo assessment of myocardial mechanical parameters in mice was determined 4 hours[2] following a single IV bolus administration of PS-341 (10 mg/kg; 30 mg/m2) or saline. While there were no statistically significant differences in heart rate, left ventricular pressure, rates of pressure development (dP/dt max) and decay (dP/dt min), and the time constant of isovolumic relaxation (tau), there appeared to be a trend toward increases in these parameters. Although significant changes in cardiac parameters were not evident, the general profile of changes in the mouse do not mirror those detected in the monkey (HR increases in the mouse were transient, up to 30 minutes post-dose, and were followed by prolonged bradycardia, which declined from 30 minutes post-dose to sacrifice). This study provides little insight into possible direct effects of PS-341 on mouse heart or the effect of PS341 in the monkey. Additional analysis involving multiple post-dosing intervals would have provided greater evidence for the assertion that based on this data there was no evidence of a direct inotropic effect on the heart.

In the sponsor's discussion of study G465502A they hypothesize that the observed changes in HR are due to a *"compensatory response to progressive hypotension caused by decreased peripheral resistance"*. Given the number of animals studied, a conclusive statement cannot be made about changes in HR, MAP and contractility. While the pattern of effects point to a change in peripheral resistance initiating changes in HR and MAP, the causality of the changes in peripheral resistance cannot be determined from these studies. Numerous physiological phenomena could account for the changes in peripheral resistance, such as antagonism of □-adrenergic receptors in vascular beds. However, the receptor pharmacology presented in , does not present an obvious receptor population on which this drug is acting. The sponsor hypothesizes that *"PS341 promoted robust expression of the prostanoid vasoactive mediators PGE_2 and PTI_2 by human aortic endothelial cells"*. While studies indicate that PGE_2 and PGF_1alpha expression is enhanced, the PS-341 concentrations utilized in the study are substantially higher than the AUC (7 fold higher) and Cmax (5 fold higher) values observed following a single dose of 1.2 mg/m^2 in monkeys.

Although increases in HR (□10-40 %, following □ 0.30 mg/kg), decreases in MAP (□ 20-100%, following □ 0.30 mg/kg) and increases in contractility (□ 50 - 400% following □ 0.03 mg/kg) were seen in the anesthetized monkey study, the company asserts there was a *" lack of toxicologically significant findings"* due to an absence of mortality. Additionally, the sponsor hypothesizes that the observed effects may *"best [be] 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"* (study KLAW- 191). However, data from the study does not appear to support the hypothesis that a decrease in body temperature promulgated the cardiac disturbances, given that the test animals were sacrificed before signs of terminal hypotension and immanent mortality were manifested.[3] Additionally, in the mouse, hypothermia (□°C) was detected following administration of

[1] The sponsor submitted additional data following completion of the consultation review by Dr. Proakis. These data were intended to address possible direct effects of PS-341 on the heart (increased contractility) A comprehensive review of these data within the division led to a conclusion that the newly submitted information did not materially effect the conclusions drawn from the previous submissions.

[2] The four hour post-dose timepoint was designated by the sponsor as the time of "maximal effect" for cardiac responses to PS-341. However, data obtained from multiple studies suggests that while cardiac changes may be seen as soon as one hour after dosing (i.e., doses of ≥ 3.0 mg/m2 in the monkey), these effects were progressive and may not have reached maximal response by twelve hours post dose. Thus, the study may be inadequate to support a conclusion that PS-341 had no direct effect on the isolated heart.

[3] It should be noted that the KLAW-191 study was performed on anesthetized animals that were sacrificed at 6 hours following PS-341 administration, whereas in the previous studies (G465502A and 6837-113; freely moving telemetry monitored animals) in which clear signs of cardiac effects and mortality were noted, animals were sacrificed at 12-14 hours post-treatment.

10mg/kg PS-341, whereas hyperthermia (1°C) was detected in the monkey. The company asserted that hypothermia was the causative factor for change in heart rate. While changes in HR were attenuated in mice with the maintenance of normal body temperature, the cardiovascular effects of PS-341 were not attenuated with the maintenance of body temperature maintenance in monkeys. While the company asserts that hypothermia was the causative factor for changes in cardiac functioning following PS-341 administration, the non-clinical data do not clearly support this perspective. Therefore, statements regarding the maintenance of body temperature as a treatment for excess drug related toxicity and cardiovascular support do not appear to be justified for inclusion in the product label.

Further studies in the mouse investigated the etiology of cardiovascular adverse events through the administration of receptor blocking agents. The co-administration of atropine and PS341 (10 mg/kg) was used to evaluate possible vagal nerve mediated effects. While it was the sponsor's assertion that atropine did not attenuate HR changes following PS-341 administration, the graphic representation of the data group mean response data appears to indicate that there was a 'trend' or 'partial' attenuation of HR effects. However, without individual data, a comprehensive an statistical evaluation of the study findings is not possible. It should also be noted that mice receiving 10mg/kg were hypokalemic (□40%) at 6.5 hours following dosing. and showed increases in ALT (100% following 3mg/kg and □ 400% following 10 mg/kg) and LDH (□ 100% following 10mg/kg); potential signs of cardiac damage or of alternative drug mediated actions of cardiovascular function. Overall, studies of the action of atropine in mice provided little insight as to the etiology of cardiovascular events following PS-341 administration.

In conclusion, given the undefined etiology of the cardiovascular effects seen in multiple non-clinical studies (changes in contractility, MAP, and HR in acute dosing studies, and cardiac necrosis with chronic dosing), and the occurrence of cardiovascular adverse events in the clinic, it appears that additional studies are warranted. Some of the 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 mg/m^2) and lethality in non-clinical studies (3.0 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 PS341 promotes dissimilar effects in monkey and mouse, future studies should be controlled to address concerns that 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 this 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.

Cardiovascular consult by Anthony Proakis, Ph.D, (Appendix A) indicates parallel conclusions..

SPECIAL TOXICOLOGY:

Dog Study: Intraprostatic Dog — **Volume 4.2.3.7.7.26.**

Conducting Laboratory and Location:

Date of Initiation: 08/03/99
GLP Compliance: NOT INDICATED, NO
Species and strain/sex: Beagle dog (coonhound)/male
Weight: ~30kg
Route: Intraprostatic injections, injections were delivered via laparotomy under general anesthesia after cystopexy by attaching the urinary bladder to anterior abdominal wall and placement of radio-opaque seeds in both prostate lobes. Subsequent injections were given IP trans-abdominal under CT guidance.

Dosing Regimen:

Regimen	#	Dose (total)	Volume
Single dose	2	Vehicle	0.5 ml injected into three locations in both lobes. Total volume = 3mL
	2	0.2 mg/kg (6 mg)	
	2	0.6 mg/kg (20mg)	
Repeated dose (biweekly X3)	6	0.13 mg/kg (4 mg/dose)	

Drug, lot #, purity: not indicated
Formulation/vehicle: normal saline

Observations:

Dose	Subject	Clinical Signs
Saline	51-816	Day 2-6: hematuria
0.2 mg/kg (single dose)	84-512	Day 2-7: hematuria
	3C0326	Day 2-7: hematuria
0.6 mg/kg (single dose)	79-712	Day 2: Anorexia, vomiting, hematochezia
		Day 6/7: **Posterior Ataxia**
	50-722	Day 2-7 hematuria
0.13mg/kg (Repeat dose)	304905	Day 6 (3rd cycle): **Complete Ataxia**
	79-706	Day 8 (1st cycle): Pitting edema
		Day 2(3rd cycle): Rectal Bleeding

Histopathology:
Listed based on nature of change with number of occurences paranthetically. Changes which are not noted singularly per group were not representative of changes in a single subject, but of changes in multiple subjects.

DOSE	Saline (n=2)	0.2 mg/kg (single) (n=2)	0.6 mg/kg (single) (n=2)	0.13 mg/kg (repeat) (n=5)
Adrenals				
Bone Marrow smear				
Bone (femur)				
Brain			Hemorrhage	
Cecum				
Colon	Lymphocytic			
Duodenum	Lymphocytic			Eosinophillic (x2) Lymphocytic Necrotic
Heart				
Ileum				
Injection site	Lymphocytic		Hemorrhage(x2)	
Jejunum				
Kidneys	Inflammation lymphocytic	Fibrosis Lymphocytic (x2)		Circulatory Hypertrophy
Liver	Generalized lesions	Fibrosis Macrophage infiltration	Inflammation	Fibrosis (x2) Necrosis
Lungs		Metaplastic Lesions	Inflammation	Fibrous Inflammation (x2)

Lymph nodes	Hyperplastic, Lymphocytic	Atropic Follicular Hyperplasia		Edema
Pancreas				
Parathyroid/Thyroid			Cystic (remnants)	Cystic (remnants)
Pituitary				
Prostate	Lymphocytic (x2) Inflammation (x2) Fibrosis (x2)	Atrophy (x2) Dilated/cystic Acini (x2) Edema (x2) Fibrosis (x2) Granulation Lymphocytic	Atrophy(x2) Dilated/ Cystic Acini (x2) Edema(x2) Fibrosis Lymphocytic (x2) Hemorrhage	Atrophy(x2) Dilated/ Cystic Acini (x5) Edema Eosinophilic Fibrous (x2) Lymphocytic (x2)
Salivary gland	Inflammation			
Spinal cord				Edema Hypertrophy/Hyperplasia of Schwann cells
Spleen	Engorged	Engorged	Engorged	Engorged
Stomach	Lymphocytic			Lymphocytic(x2)
Testes			Cryptorchid (R)	
TONSILS	Hyperplastic		Hyperplastic Lymphoid	Hyperplastic Lymphoid
Urinary bladder	Hemorrhage	Granulation Hemorrhage	Hemorrhage Edema Granulation	Lymphocytic (x2) Granulation

Most notable, histopathologic examination of multiple sections of the spinal cord from the animal which received 0.13 mg/kg PS-341 (repeat dose schedule) revealed:

- Separation by edema of the nerve fibers of the ventral nerve roots.
- Myelin sheath thinning and fragmentation
- Basophilic axons and myelin sheath containing basophilic bodies suggestive of nuclear fragments.
- Schwann cell hypertrophy and hyperplasia of the lumbar spinal cord.
- No significant lymphocytic inflammation within spinal nerve roots, meninges or spinal cord sections

Examination of the dog receiving a single dose of PS341 did not reveal histopathologic anomalies in the spinal cord. The company asserts that this breed of dogs is particularly susceptible to acute idiopathic polyradiculoneuritis, or Coonhound paralysis. However this condition is known to be a rare condition most frequently associated with a bite from a raccoon. The clinical course and histopathology described appears somewhat different from coonhound paralysis, which usually has significant lymphocytic inflammation. 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 company 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.

Overall Summary of Intraprotstatic Injection of PS-341:

Notable changes were detected following intrapostatic injection of PS341. Most notable is the apparent retrograde degeneration from the neural plexus of the prostate to the ventral roots of the spinal cord (Wallerien degeneration) This affect is of concern particularly given the local route of administration. Previous reviews had noted a potential for neurotoxicity when administered systemically as observed by hypoactivity, and labored breathing. Clinically, neuropathies have been of major concern. These data indicate that local administration of PS341 is contraindicated, particularly when administered in areas of intense neuronal innervation.

(2) Partition of PS-341 Between Plasma and Red Blood Cells in the Cynomolgus Monkey and Sprague- Dawley Rat. Module 4, rpt-00097.

Key study findings: The concentration of [^{14}C]-PS-341 in plasma was higher (~40%) than that in RBC from rats. There was no significant difference in monkey.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation: August 13, 2002
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341 (68 μCi/mg), lot # 2619-117
Formulation/vehicle: [^{3}H]-H_2O (1 μCi/mg, control), normal saline

Methods: [^{14}C]-PS-341 (10 μM) was incubated with fresh blood samples from 3 cynomolgus monkeys and 3 Sprague-Dawley rats at 37^{o}C for 1 hour and the radioactivity in whole blood, plasma and RBC was measured.

Results: Partition of PS-341 (mean average of three animals) between plasma and red blood cells in rats and monkeys.

Species	Animal #	[^{14}C]-PS-341 / [^{3}H]-H_2O			Plasma/RBC
		Whole blood	Plasma	RBC	
Rat	1	1.29	1.07	0.72	1.47
	2	1.17	1.18	0.84	1.40
	3	1.25	1.11	0.78	1.42
Monkey	1	1.04	1.11	0.96	1.15
	2	1.12	1.12	1.03	1.09
	3	1.19	1.05	0.87	1.21

(3) Partition of PS-341 Between Human Red Blood Cells and Plasma. Module 4, rpt-00024.

Key study findings: The concentration of PS-341 in plasma was slightly higher (~30%) than in human RBC.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation: February 5, 2002
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341 (68 μCi/mg), lot # 2619-117
Formulation/vehicle: [^{3}H]-H_2O (1 μCi/mg, control), normal saline

Methods: [^{14}C]-PS-341 (10 µM) or [^{3}H]-H_2O was incubated with fresh human blood samples (3 donors) at 37^{O}C for 1 hour and the radioactivity in whole blood, plasma and RBC was measured.

Results: Partition of PS-341 (mean average) between human plasma and red blood cells.

Donor	[^{14}C]-PS-341 / [^{3}H]-H_2O			Plasma/RBC
	Whole blood	Plasma	RBC	
1	1.10	1.09	0.82	1.34
2	1.11	1.08	1.03	1.05
3	1.12	1.03	0.83	1.25

(4) The In Vitro *Protein Binding* of [^{14}C]-PS-341 to Plasma from Rat, Monkey, and Human. Module 4, 6837-101.

Key study findings: The binding of ^{14}C-PS-341 to plasma proteins was 72% in monkey, 83% in human and 85% in rat.

Conducting laboratory and location: [ ]

Date of study initiation: May 17, 1997
GLP compliance: Yes
QA report: yes (X) no ()
Drug, lot #, radiolabel, and % purity: ^{14}C-PS-341, lot # MDL 10842-06 and PS-341 lot #5, purity not mentioned.

Methods: The samples were incubated for 10 minutes at 37^{0}C and the extent of binding was determined by ———

Results:

Percent of ^{14}C-PS-341 bound to rat, monkey and human plasma.

Concentration [^{14}C]-PS-341 (µg/mL)	Rat		Monkey		Human	
	Mean Percent Bound	SD	Mean Percent Bound	SD	Mean Percent Bound	SD
0.01	81.6	11.7	67.2	8.47	78.9	2.49
0.03	86.1	0	72.7	0.404	80.6	0.656
0.01	85.3	3.32	73.8	1.92	85.7	1.57
0.3	87.3	0.819	74.8	0.300	85.4	0.451
1	83.9	0.153	73.3	0.208	84.0	0.0577
Overall Mean	84.9		72.4		82.9	
Overall SD	5.04		4.29		3.00	

SD Standard deviation.

The percentage biding of ^{14}C-PS-341 to plasma proteins was independent of PS-341 concentration from 0.01 to 1 µg/mL.

Distribution

(1) ^{14}C-MDL 108421: Quantitative Whole Body Autoradiography in male rats after a single intravenous administration of approx. 0.3 mg/kg body weight. Module 4, tep-233-1.

Key study findings: [redacted]

Conducting laboratory and location: [redacted]

Date of study initiation:	February 18, 1997
GLP compliance:	Yes
QA report:	yes (X) no ()
Drug, lot #, radiolabel, and % purity:	[U-^{14}C]-MDL 108421 (PS-341), lot # Z 27020-0, 1010 MBq/g, — purity [labeled at phenylalanine]
Formulation/vehicle:	Saline

Dosing:

Species/strain:	Sprague Dawley, CD, C57BL/6, B/C rats
#/sex/group or time point (*main study*):	5 males
Satellite groups used for toxicokinetics or recovery:	No
Age:	6-10 weeks
Weight:	194-211 g
Doses in administered units:	0.3 mg/kg
Route, form, volume, and infusion rate:	Intravenous bolus into a tail vein

Results:

Concentrations (µg equivalents/g) in organs and tissues after intravenous administration of approximately 0.3 mg/kg body weight to male rats are shown below.

Organs/ Tissue	0.167 h	1 h	3 h	6 h	24 h
Adrenal	1 40	1.55	1.68	1.38	0 69
Blood	0.33	0.15	0 19	0.16	0.20
Bone marrow	0 37	0 36	0.54	0.39	0.25
Brain	LOQ	LOQ	LOQ	LOQ	LOQ
Brown fat	0.36	0.26	0.20	0.22	0.17
Kidney	1.16	0.72	0 73	0.57	0.39
Lacrimal gland	0.39	0.42	0.50	0.40	0.30
Liver	0.96	0.63	0.65	0.51	0.51
Lung	0.61	0.28	0.26	0.23	0.25
Myocardium	0 43	0.30	0.35	0.25	0 18
Pancreas	0.61	0.55	0.61	0.52	0.39
Salivary gland	0.50	0.56	0 63	0.45	0 43
Skeletal muscle	0.23	0.15	0.17	0.16	0.15
Spinal cord	LOQ	LOQ	LOQ	LOQ	LOQ
Spleen	0.81	0.63	0.74	NS	0 80
Testis	LOQ	LOQ	LOQ	LOQ	LOQ
Thymus	0.16	0 15	0.23	0.21	0.24
Thyroid	0 69	0 43	0 47	0 42	0.28

The radioactivity concentrations in gastrointestinal tract and the urinary bladder were not determined due to their inhomogeneous distribution. The radioactivity in the brain and spinal cord was below the limit of quantification ——— ndicating that no or minor penetration of the blood brain barrier took place.

(2) Biliary Excretion of PS-341 in Male Rats Following a Single Intravenous Dose of [^{14}C]-PS-341. Module 4, 6837-114.

Key study findings: Slow biliary excretion was the primary route of elimination of [^{14}C]-PS-341 derived radioactivity in bile duct-intact and bile duct-cannulated rats.

Conducting laboratory and location: [ ]

Date of study initiation: July 30, 1997

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: [U-^{14}C]-PS-341, lot # MDL 108421-08, (27.4 µCi/mg), — purity.
PS-341, lot # 5, — purity.

Formulation/vehicle: Saline

Methods: Groups 2 and 3 animals were previously cannulated in the bile duct and duodenum by the supplier ———.

Dosing:

Species/strain:	Rats [Hla: (SD) CVF]
#/sex/group or time point (main study):	See below
Satellite groups used for toxicokinetics or recovery:	None
Age:	9 weeks
Weight:	291-329 g

Doses in administered units:

Group	Number of Males	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)
1	4	0.2	1
2	4	0.2	1
3[a]	1	0.5	2.5

Group 1 – bile duct-intact animals
Group 2 – bile duct-cannulated animals
a - Samples collected from this animal (Group 3) were used for method development purposes only.

Route, form, volume, and infusion rate: Bolus intravenous via lateral tail vein

Observations and times:

Clinical signs: Group 3 animal was hypoactive, hunched and have labored breathing on study day 2.

Urine, feces and bile samples were collected as shown below.

Group	Number of Males	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)		Collection Times (Hours Postdose)
1	4	0.2	1	Urine:	predose, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120
				Feces:	predose, 0-24, 24-48, 48-72, 72-96, and 96-120
2	4	0.2	1	Urine:	predose, 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72
				Feces:	predose, 0-24, 24-48, and 48-72
				Bile:	predose, 0-2, 2-4, 4-8, 8-12, 12-24, 24-48, and 48-72
3[a]	1	0.5	2.5	Urine:	0-4, 4-8, 8-12, and 12-24
				Feces:	0-24
				Bile	0-2, 2-4, 4-8, 8-12, and 12-24

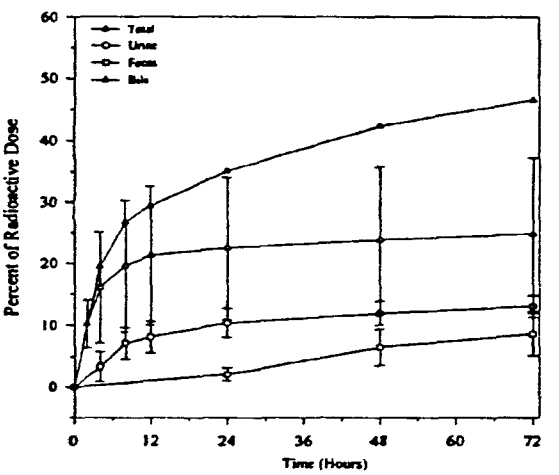
a Urine, feces, and bile collected from the Group 3 animal were used for metabolite profiling method development purposes only.

Results:

Mean cumulative percent of radioactive dose in urine and feces at specified intervals postdose for male rats (Group 1) following a single intravenous dose of [^{14}C]-PS-341 (0.2 mg/kg) are shown below.

In bile duct-intact animals, most of the radioactivity was recovered in the feces (40.4%). Approximately 12% was recovered in urine.

Mean cumulative percent of radioactive dose in urine, feces, and bile at specified intervals postdose for male rats (Group 2) following a single intravenous dose of [^{14}C]-PS-341 are shown below.



In the bile duct-cannulated animals, approximately 25% of the radioactivity was recovered in the bile, 9% in the feces and 13% in the urine.

The residual carcasses at 72 hours postdose retained a significant amount of dosed radioactivity in both the bile duct-intact (37%) and bile duct-cannulated (42%) animals. The overall recovery of radioactivity was approximately 91% in both the bile duct-intact and bile-duct-cannulated animals

(3) Tissue Distribution, Biliary Excretion, and Mass Balance of ^{14}C-PS-341 in Male Sprague Dawley Rats Following a Single Bolus Intravenous Dose. Module 4, pk-800.

Key study findings: The radioactivity recovered by 72 hours postdose was 39% in the feces of non-cannulated animals and 35% in the bile of cannulated animals.

Conducting laboratory and location: [redacted]
Date of study initiation: November 13, 2001
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, lot # 2619-117, (68 µCi/mg), 95% purity.
Formulation/vehicle: Sterile saline with 5% ethanol.

Dosing:

Species/strain: Rattus norvegicus (rat) / Crl:CD(SD)IGS BR
#/sex/group or time point (main study): Males
Satellite groups used for toxicokinetics or recovery: None
Age: 6-7 weeks
Weight: 178-265 g
Doses in administered units:

Group No.	n	Description of Animal	Nominal Dose (mg/kg)	Route of Admin	Nominal Conc (mg/mL)	Nominal Conc (µCi/kg)	Dose Volume (mL/kg)	Sacrifice Time (hours)	Group Designation
1	12	Albino male	0.25	iv	0.05	17	5	1, 24, 48, and 72[a]	QTD/MB
2	8	Albino male	0.25	iv	0.05	17	5	1, 24, 48, and 72	QWBA
3	4	Albino male	0.25	iv	0.05	17	5	72	BE/MB

[a] Determination of mass balance on 72-hour animals only.

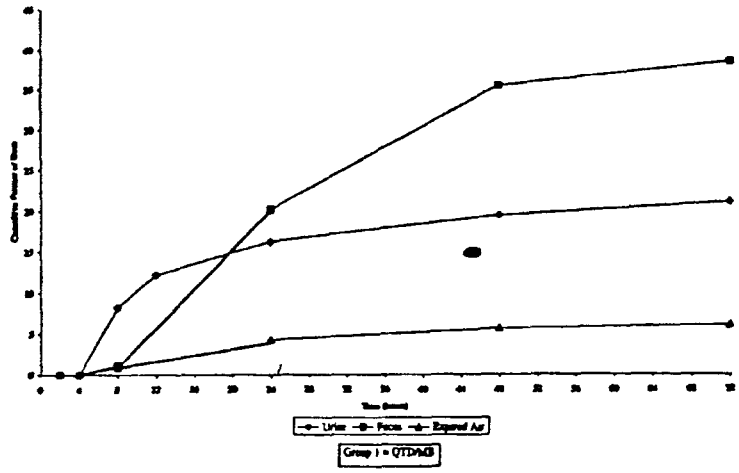
Group 1 was designated to determine quantitative tissue distribution (QTD) by excision and mass balance (MB).
Group 2 was designated for quantitative whole body autoradiographic analysis (QWBA) of tissue distribution over time.
Group 3 was designated to biliary excretion and mass balance (BE/MB).

Route, form, volume, and infusion rate: Intravenous bolus via lateral tail vein.

Observations and times: Samples were collected for quantification of ^{14}C as shown below.

Name	Time collected (hr) postdose
Urine	0-8, 8-12, 12-24, 24-48, and 48-72
Feces and expired air trapping	0-8, 8-24, 24-48, and 48-72
Bile	0-2, 2-4, 4-8, 8-12, 12-24, 24-48, and 48-72

Mean cumulative percent of radioactivity in excreta at specified times postdose determined in non-cannulated male Sprague Dawley rats (Group 1) following single intravenous administration (0.25 mg/kg) of ^{14}C-PS-341.



The mass balance of radioactivity was 102.9±1.3%, with 39% in feces, 21% in urine and 6% in expired air. The excised tissues/homogenates accounted for 15% of the recovered dose, while 21% remained in the dissected carcass at 72 hours postdose.

Group 2

Concentration of radioactivity (µg equiv/g) in blood and plasma at specified times postdose.

Group	Time (hour)	Blood	Plasma
2	1	0.14	0.01
	24	0.11	0.005
	48	0.09	0.00
	72	0.08	0.00

All samples reached their highest concentration at 1 hour postdose.

The distribution of radioactivity to tissues after single IV administration of PS-341 was wide spread (Group 2). At 72 hours postdose, the ^{14}C-PS-341 derived radioactivity concentrations were approximately half that of the 1 hour levels. The excision data shown above complemented the autoradiographic derived distribution data. According to the sponsor, quantitative whole body autoradiographic analysis was able to provide in some tissues a more detailed localization of the radioactivity than the tissue excision method.

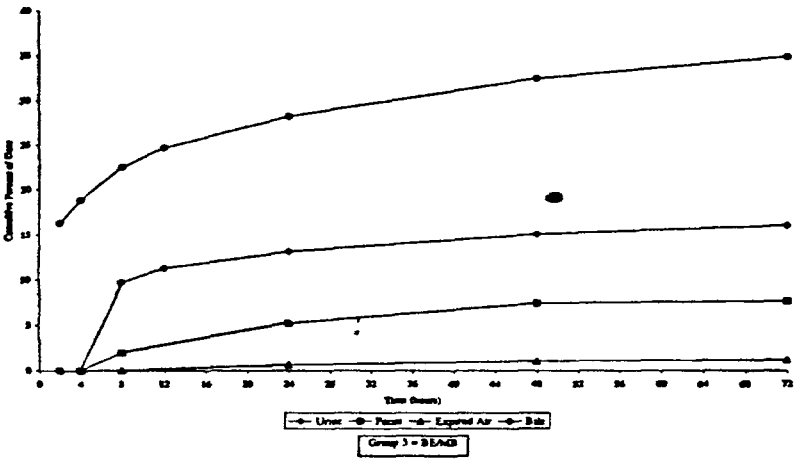
Group 3

Concentration of radioactivity (µg equiv/g) in blood and plasma at specified times postdose.

Group	Time (hour)	Blood	Plasma
3	72	0.13	0.01

All samples reached their highest concentration at 1 hour postdose.

Mean cumulative percent of radioactivity in excreta at specified times postdose determined in cannulated male Sprague Dawley rats (Group 3) following single intravenous administration (0.25 mg/kg) of ^{14}C-PS-341 are shown below.



The radioactivity recovered was 35% in bile, 32% in carcass, 16% in urine, 8% in feces, and 1% in expired air. Total radioactivity recovery was 93±6%.

(4a) Biliary Excretion of PS-341 in Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341. Module 4, 6837-116.

Key study findings: Urine was the primary route of excretion in both bile duct-intact (Group 1) and bile duct-cannulated (Group 2) male cynomolgus monkeys.

Conducting laboratory and location:	———
Date of study initiation:	September 9, 1997.
GLP compliance:	Yes
QA report:	yes (X) no ()
Drug, lot #, radiolabel, and % purity:	[^{14}C]-PS-341, lot # MDL 108421-08, — purity. PS-341, lot # 5, — purity.

Dosing:

Species/strain:	Cynomolgus monkeys
#/sex/group or time point (main study):	Group 1 - 2 males (bile duct-intact) and Group 2 - 2 males (bile duct-cannulated)
Satellite groups used for toxicokinetics or recovery:	None
Age:	Not mentioned
Weight:	3.1 to 3.6 kg
Doses in administered units:	0.2 mg/kg (~4.7µCi/kg)

Route, form, volume, and infusion rate: Intravenous bolus via saphenous vein

Observations and times: Blood samples were collected from groups 1 and 2 animals at predose, 5,10, 30 min, and 1, 2, 4, 8, 12, and 24 hours after dosing.

Urine samples were collected from all animals at predose, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hours after dosing.

Feces were collected from all animals at predose, 0-24, 24-48, 48-72, 72-96, and 96-120 hours after dosing.

Bile was collected from the Group 2 animals predose, and at 0-2, 2-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hours postdose.

Results:

Concentration of Radioactivity in Plasma at Specified Times Postdose for Groups 1 (bile duct-intact) and 2 (bile duct-cannulated) Male Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341 (0.2 mg/kg) is shown below.

	ng Equivalents [^{14}C]-PS-341/g					
	Group 1			Group 2		
Collection	Animal Number			Animal Number		
Time	104618	1046321	Average	104619	104623	Average
5 minutes			265			246
10 minutes			159			163
30 minutes			99.7			110
1 hour			73.9			80.7
2 hours			54.8			58.7
4 hours			51.1			50.7
8 hours			52.1			42.3
12 hours			47.9			39.8
24 hours			55.7			43.2

The average maximum concentration of radioactivity in plasma was observed at 5 minutes postdose in both groups. The average concentration declined slowly up to 4 hours and maintained at this level through 24 hours postdose, the last sampling time. The differences between Groups 1 and 2 at 4-8 hours (↓ in group 2, about constant for group 1) may indicate enterohepatic recirculation.

Percent of Radioactivity in Tissues at 120 Hours Postdose for Group 1 (bile duct-intact) Male Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341.

	Percent of Radioactive Dose		
	Animal Number		
Sample	104618	104621	Average
Adrenals			0.02
Bone (femur)			<0.005
Bone marrow			0.01
Brain			0.31
Fat (abdominal)			0.04
Kidneys			0.77
Liver			5.64
Muscle (thigh)			0.61
Prostate			0.05
Small intestine (distal)			0.13
Small intestine (proximal)			0.02
Spleen			0.43
Total			8.04

ND Not detectable.

The average recovery was 8% of the administered radioactivity. The recovery was highest in the liver (5.6%), kidneys (0.8%), muscle (0.6%), spleen (0.4%) and brain (0.3%). Radioactivity in the heart was not measured.

Tissue:Plasma Ratios for Concentration of Radioactivity for Group 1 Male Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341 (0.2 mg/kg) are shown below.

	Tissue:Plasma Ratios		
	Animal Number		
Sample	104618	104621	Average
Adrenals	—	—	5.63
Blood	—	—	2.35
Bone (femur)	—	—	0.037
Bone marrow	—	—	2.46
Brain	—	—	0.766
Fat (abdominal)	—	—	0.795
Kidneys	—	—	10.3
Liver	—	—	11.2
Muscle (thigh)	—	—	2.60
Prostate	—	—	2.27
Small intestine (distal)	—	—	4.07
Small intestine (proximal)	—	—	3.45
Spleen	—	—	13.8

ND Not detectable.

The average tissue:plasma concentration ratio were greater than one indicating rapid elimination of drug-derived radioactivity from the plasma. The presence of [^{14}C]-PS-341 in brain suggest possibility of toxicity to CNS, unlike rat where no radioactivity in this compartment was detected (see study PK 800).

Percent of Radioactive Dose in Urine, Feces, Bile, Cage Wash, and Cage Wipe at Specified Intervals Postdose for Groups 1 and 2 Male Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341 (0.2 mg/kg) are shown below.

	Percent of Radioactive Dose					
Collection Intervals	Group 1			Group 2		
	Animal Number			Animal Number		
(Hours)	104618	1046321	Average	104619	104623	Average
	Total Excreta (Urine, Feces, and Bile)					
0-24	—	—	14.1	—	—	26.4
24-48	—	—	3.81	—	—	4.55
48-72	—	—	3.60	—	—	4.03
72-96	—	—	4.32	—	—	3.37
96-120	—	—	2.98	—	—	2.58
Subtotal	—	—	28.8	—	—	40.9
	Cage Wash and Cage					
120+	—	—	4.44	—	—	3.91
120+	—	—	0.98	—	—	0.72
Total	—	—	34.2	—	—	45.5

NA Not applicable.
NS No sample.
a Cage wash.
b Cage wipe.
Note: Total excreta (urine, feces, and bile) values are calculated from rounded group values.

In group 1 (bile duct-intact) animals, urine and feces accounted for 21% and 8% of the dosed radioactivity, respectively. In group 2 (bile duct-cannulated) animals, urine, bile and feces accounted for 21%, 17% and 3% of the dosed radioactivity, respectively.

Pharmacokinetic Parameters far Radioactivity in Plasma for Groups 1 and 2 Male Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341.

Animal Number	Dose Administered (mg/kg)	Terminal $t_{1/2}$ (hours)[a]	β (hours)	$AUC_{0-\infty}$ (ng equiv·hour/g)	Plasma Clearance (g/hour/kg)[b]	Volume of Distribution (mL/kg)[c]
104618	0.168	359	0.0019332	26745	6.28	3249
104621	0.171	155	0.0044845	15267	11.20	2498
104619	0.173	99	0.0070201	6002	28.82	4106
104623	0.173	101	0.0068546	8785	19.69	2873
Mean	0.17	137	0.0051	14200	16.50	3181
SD	0.002	NA	0.00239	9220	9.91	688

equiv Equivalents.
a Harmonic mean = 0.693/mean β.
b Clearance = (Dose mg/kg x 1000000 ng/mg)/$AUC_{0-\infty}$
c Volume of Distribution = Clearance/β.
SD Standard deviation.
NA Not applicable.

The $t_{1/2}$ values were long and variable for both groups 1 (155 and 359 hours) and 2 (99 and 101 hours).

PS-341 was extensively metabolized to several unknown metabolites and no unchanged PS-341 was detected in bile and urine samples.

(4b) **Addendum to ——6837-116:**
20S PROTEASOME INHIBITION AND TISSUE SAMPLE ANALYSIS REPORT. Module 4, 6837-116 addendum.

Key study findings: The [^{14}C]-PS-341 metabolites in bile and urine of bile duct cannulated monkeys did not show 20S-proteasome activity 5 days after a single dose administration.

Conducting laboratory and location: ——
Date of study initiation: September 9, 1997.
GLP compliance: Yes
QA report: yes (X) no ()
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, lot # MDL 108421-08, —purity.
PS-341, lot # 5, — purity.

Methods: ——

Dosing:

Species/strain:	Cynomolgus monkeys
#/sex/group or time point (main study):	2 males (bile duct-cannulated)
Satellite groups used for toxicokinetics or recovery:	None
Age:	Not mentioned
Weight:	3.1 to 3.6 kg
Doses in administered units:	0.2 mg/kg
Route, form, volume, and infusion rate:	Intravenous bolus via saphenous vein

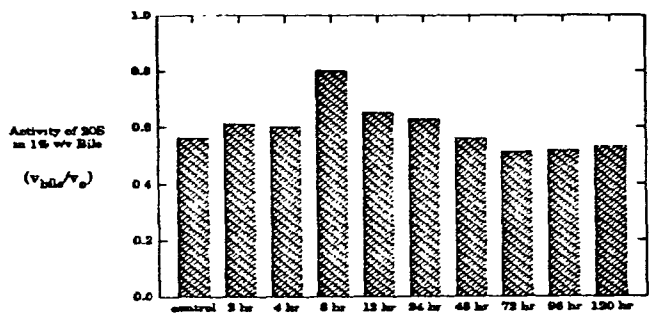
Observations and times:

Urine samples were collected from all animals at predose, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hours after dosing.

Bile was collected from the Group 2 animals predose, and at 0-2, 2-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hours postdose.

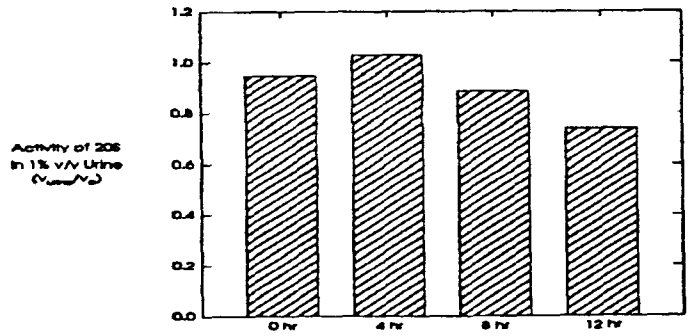
Results:

Inhibition of 20S Proteasome Activity in Bile Samples Obtained from Cannulated Cynomolgus Monkeys Following Intravenous Administration of [^{14}C]-PS-341.



There were no differences in 20S-proteasome inhibition in predose (control) and dosed samples.

Inhibition of 20S Proteasome Activity in Urine Samples Obtained from Cannulated Cynomolgus Monkeys Following Intravenous Administration of [^{14}C]-PS-341.



All urine samples tested showed 20S-proteasome activity but there were no differences between the predose (control) samples and samples collected up to 12 hours after dosing.

(5) Amended Final Report:
Tissue Distribution and Mass Balance of Total Radioactivity in Male and Female Cynomolgus Monkeys Following a Single Bolus Intravenous Dose of ^{14}C- PS- 341. Module 4, pk-888.

Key study findings: The distribution of radioactivity in the male monkeys was comparable to that seen in female monkeys. The frozen tissue excision data indicate that brain contained small amount of activity as compared to liver (1.4%). The fecal and renal routes were indicated for male and female monkeys, respectively, as elimination pathways.

Conducting laboratory and location: [ ]

Date of study initiation: April 16, 2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, lot # 2619-117 (69 µCi/mg), purity.
Formulation/vehicle: Sterile saline containing 5% ethanol

Dosing:

Species/strain: Macaca fascicular, cynomolgus monkey
#/sex/group or time point (main study): 4
Satellite groups used for toxicokinetics or recovery: None
Age: 16-22 months
Weight: 1.8 –2.2 kg

Doses in administered units: 0.2 mg/kg

Group No.	#	Description of Animal	Nominal Dose (mg/kg)	Route of Admin	Nominal Concentration (mg/mL)	Nominal Concentration (µCi/kg)	Dose Volume (mL/kg)	Sacrifice Time (hours)
1	4	Male	0.2	iv	0.2	13.6	1	1, 24, 72, and 144
2	4	Female	0.2	iv	0.2	13.6	1	1, 24, 72, and 144
3	2	One Male and One Female	0.2	iv	0.2	13.6	1	144[a]

[a] Following collection of urine, cage rinse, and feces.
iv = intravenous

One male and one female from groups 1 and 2 were sacrificed at 1, 24, 72, and 144 hours postdose. Blood and tissue samples were collected from each monkey to determine tissue distribution (Groups 1 & 2).

Urine and feces were collected from group 3 animals every 24 hours until 144 hours post dose for mass balance determination.

Route, form, volume, and infusion rate: Intravenous bolus by cephalic vein

Results:

Whole Blood, Plasma, and Red Blood Cells Concentrations (µg equiv/ g) of Total Radioactivity in Male and Female Cynomolgus Monkeys Following a Single Intravenous Administration of ^{14}C- PS- 341 at a Target Dose Level of 0.2 mg/ kg.

	Time Point/Animal No.									
	Male					Female				
	1 h	24 h	72 h	144 h	144 h	1 h	24 h	72 h	144 h	144 h
Sample	001M	002M	003M	004M	005M	006F	007F	008F	009F	010F
Blood (whole)										
Plasma										
Red blood cells										
Blood Plasma Ratio										

LOQ for LSC-derived concentrations was ~~~ µg equiv/g

The maximum plasma concentration was observed at 1 hour postdose. The radioactivity in blood, plasma and red blood cells was similar in male and female animals. RBC (cellular portion of the blood) showed higher radioactivity than plasma.

Tissue Concentrations (µg equiv/g) of Total Radioactivity in Male and Female Cynomolgus Monkeys at 144 hours Following a Single Intravenous Administration.

	Animal No.	
Tissue	005M (Male)	010F (Female)
Heart		
Kidney		
Liver		
Lung		
Pancreas		

LOQ for LSC-derived concentrations was ~~~

The majority of tissues reached C_{max} at 1 hour postdose (the earliest time point) and then declined slowly. The majority of the radioactivity was associated with the major organs of excretion. Low concentrations of radioactivity remained measurable in the majority of the sample tissues including heart at 144 hours postdose.

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Tissue Concentrations (µg equiv/g) of Total Radioactivity in Male and Female Cynomolgus Monkeys Following a Single Intravenous Administration of ^{14}C- PS- 341 at a Target Dose Level of 0.2 mg/ kg are shown below.

	Time Point/Animal No.			
	Male		Female	
	1 hour	72 hour	1 hour	72 hour
Tissue	001M	003M	006F	008F
Brain (cerebrum)	0.011	0.005	0.013	0.003
Liver	0.803	0.320	0.952	0.232

The frozen tissue excision data indicate that brain contained small amount of activity as compared to liver.

Excretion of Total Radioactivity in Male Cynomolgus Monkey (Group 3) Following a Single Intravenous Administration of ^{14}C- PS- 341 at a Target Dose Level of 0.2 mg/ kg.

Results Expressed as Percent Administered Dose

	Time Interval (h)						
Sample	0–24	24–48	48–72	72–96	96–120	120–144	Total
Urine	17 77	3 06	1 78	1 02	0 86	0 85	25 34
Cage rinse	3.26	1.58	2.30	0.58	0.55	2.84	11.11
Feces	0.43	5 27	3.96	1.66	0 89	0.75	12 96
Cage debris	BLQ	NS	BLQ	NS	NS	BLQ	0.00
GI tract	—	—	—	—	—	3.16*	3.16
Heart	—	—	—	—	—	0 15*	0 15
Kidney	—	—	—	—	—	0.65*	0.65
Liver	—	—	—	—	—	4.12*	4.12
Lung	—	—	—	—	—	0 49*	0 49
Pancreas	—	—	—	—	—	0.10*	0.10
Carcass	—	—	—	—	—	21.52*	21.52
Total	21 46	9 91	8 04	3 26	2 30	34 63	79 60

* Excised tissues and carcass were collected at sacrifice (144 hours).

NS indicates no sample

BLQ indicates sample below limit of quantification (LOQ [redacted])

— indicates not applicable.

Excretion of Total Radioactivity in Female Cynomolgus Monkey (Group 3) Following a Single Intravenous Administration of [^{14}C]- PS- 341 at a Target Dose Level of 0.2 mg/kg. Results Expressed as Percent Administered Dose are Shown Below.

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Sample	Time Interval (h)						Total
	0–24	24–48	48–72	72–96	96–120	120–144	
Urine	3.07	2.44	1.57	1.20	1.31	1.00	10.59
Cage rinse	7.48	0.89	2.06	1.23	0.32	3.09	15.07
Feces	3.37	5.90	2.41	1.56	1.02	0.68	14.94
Cage debris	BLQ	BLQ	BLQ	NS	BLQ	BLQ	0.00
GI tract	—	—	—	—	—	3.76*	3.76
Heart	—	—	—	—	—	0.10*	0.10
Kidney	—	—	—	—	—	0.49*	0.49
Liver	—	—	—	—	—	3.27*	3.27
Lung	—	—	—	—	—	0.35*	0.35
Pancreas	—	—	—	—	—	0.07*	0.07
Carcass	—	—	—	—	—	16.40*	16.4
Total	13.92	9.23	6.04	3.99	2.65	29.21	65.04

* Excised tissues and carcass were collected at sacrifice (144 hours).
NS indicates no sample.
BLQ indicates sample below limit of quantification (LOQ was ——→
— indicates not applicable

The distribution of radioactivity in the male monkeys was comparable to that seen in female monkeys. In the male monkey approximately 25 % of the radioactivity was recovered in the urine and approximately 13 % was recovered in the feces by 144 hours postdose. In the female monkey, approximately 11% was recovered in the urine and approximately 15% was recovered in the feces.

Metabolism

(1) *In Vitro* Metabolic Stability of PS- 341 in Mouse and Human Microsomes. Module 4, rpt-00012.

Key study findings: PS-341 was relatively more stable in human microsomes than in mouse microsomes.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation: May 3, 2001
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: PS-341, lot # 5A, purity not mentioned.
Formulation/vehicle: Verapamil and propranolol were used as positive controls.

Results:

Metabolism of PS-341 in Human and Mouse Liver Microsomes

	Human Microsomes[a] (% Remaining)		Mouse Microsomes[a] (% Remaining)	
Compound	15 min	30 min	15 min	30 min
PS-341	80.6	62.7	44.1	16.8
[ ]	74.4	38.6	27.7	7.3
	96.3	77.8	60.3	38.1

a: n=2 (with and without the NADPH regeneration system).

Hepatic clearance of PS-341 in mice may be higher than in humans.

(2) Preliminary Identification of PS-341 Biliary Excreted Metabolites in Rats Treated With a Single Intravenous Bolus Dose of [^{14}C]-PS-341. Module 4, rpt-00009.

Key study findings: PS-341 was metabolized into M1 to M15 (glutathione conjugates) by rats.

Conducting laboratory and location: Millennium Pharmaceuticals, Cambridge, MA., [ ]

Date of study initiation: No provided
GLP compliance: Not mentioned
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, 68 µCi/mg, purity not mentioned
Formulation/vehicle: Sterile saline containing 5% ethanol.

Methods: As a supplement to the *in vivo* samples (see below), PS-341 and its degradants A (M1), B (M2), C (M3), and D (M4) were incubated with rat liver microsomes in the presence of glutathione and/ or rat glutathione-S-transferases. The resultant metabolites helped to characterize PS-341 biotransformation pathways.

Dosing:

Species/strain:	Rat
#/sex/group or time point (main study):	Males only
Satellite groups used for toxicokinetics or recovery:	None
Age:	Not provided
Weight:	Not provided
Doses in administered units:	0.25 mg/kg
Route, form, volume, and infusion rate:	Intravenous

Observations and times: Bile samples were collected 0-2 hours postdose.

Results:

The major metabolites detected in rat bile were M1, M2, M10, M14, and M15 (glutathione conjugate).

(3) Identification and Characterization of PS-341 Metabolites in Sprague- Dawley Rats. Module 4, rpt-00099.

Key study findings: M1-M8, M23-M28, and two additional metabolites were detected in the 0.5 hour pooled plasma samples.

Conducting laboratory and location: [redacted] and Millennium Pharmaceuticals, Inc. MA.

Date of study initiation: Not provided

GLP compliance: Not mentioned

QA report: yes ( ) no (X)

Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, lot # 2619-117 (69.2 µCi/mg), purity not mentioned.

Formulation/vehicle: Sterile 0.9% sodium chloride plus 2% DMSO

Dosing:

Species/strain:	Sprague-Dawley rats
#/sex/group or time point (main study):	4 males
Satellite groups used for toxicokinetics or recovery:	None
Age:	Not provided
Weight:	Unknown
Doses in administered units:	2 mg/kg (68 µCi/mg)
Route, form, volume, and infusion rate:	Intravenous bolus

Results:

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(4) Identification and Characterization of PS-341 Metabolites in Cynomolgus Monkeys. Module 4, rpt-00119.

Key study findings: A single peak corresponding to M4 was detected in the 60 minute postdose plasma samples.

Conducting laboratory and location: ——— and Millennium Pharmaceuticals, Inc., Cambridge, MA.

Date of study initiation: Not provided

GLP compliance: No

QA report: yes ( ) no (X)

Drug, lot #, radiolabel, and % purity: [^{14}C]-PS-341, lot # 2619-117 (69.2 µCi/mg), purity not mentioned.

Methods: Plasma, urine, and feces samples from animals were analyzed by ——— and ——— techniques.

Dosing:

Species/strain:	Cynomolgus monkeys
#/sex/group or time point (main study):	Total 2 animals (1 ♂ and 1 ♀)
Satellite groups used for toxicokinetics or recovery:	None
Age:	Not provided
Weight:	Not provided
Doses in administered units:	0.2 mg/kg, 69.2 µCi/mg
Route, form, volume, and infusion rate:	Intravenous bolus

Results:

Estimated Concentrations of PS-341 and Circulating Metabolites 60 Minutes after Dose Administration

Compound	Plasma Concentration (pg/mL)
PS-341	4500
M1	700
M2	630
M3	500
M4	3300
M5	810
M6	510
M7	310
M8	44
M23	18
M24	< LOD[a]
M25	< LOD
M26	68
M27	< LOD
M28	ND[b]
M29	320
M30	120

a: Intensity less than the limit of detection (signal-to-noise ratio = 3) by visual inspection.
b. Not detected

M4 was the major metabolite observed in plasma.
Several peaks on radiochromatograms were observed in pooled urine samples.
Metabolites M1, M2, and M15 were observed in monkey fecal samples.

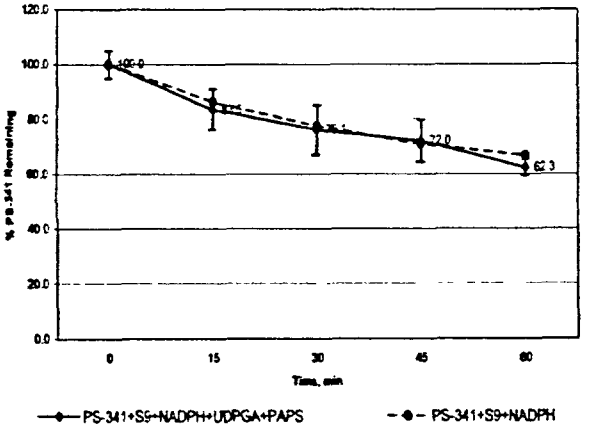
(5) In Vitro Metabolic Stability of PS-341 in Human Liver S9 Fraction. Module 4, rpt-00019.

Key study findings: The addition of phase II (conjugation) factors for glucuronidation and sulfation during incubation did not effect the metabolic stability of PS-341.

Conducting laboratory and location:	Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation:	Not mentioned
GLP compliance:	No
QA report:	yes ( ) no (X)
Drug, lot #, radiolabel, and % purity:	PS-341, lot # 5a. [^{14}C]-PS-341, lot # TCH-2591-51-B, purity not mentioned.

Results:

Metabolic Stability in Human Liver S9 Fraction (5 mg/mL) with and without Glucuronidation and Sulfation Phase II Cofactors, UDPGA and PAPS.



Phase II glucuronidation and sulfation pathways contributed very little, if any, to the metabolic degradation of PS-341.

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(6) Evaluation of Cytochrome P450 Induction Potential of PS-341 Using Cultured Human Hepatocytes. Module 4, rpt-00021.

Key study findings: PS-341 up to 50μM did not induce CYP 1A2 and CYP 3A4 activities in primary cultured hepatocytes from two human donors.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation: November 1, 2001
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: PS-341, lot # 2619-21.
Formulation/vehicle: Positive controls were omeprazole (CYP 1A2) and rifampin (CYP 3A4).

Methods: CYP 1A2 and CYP 3A4 activities were measured 48 hours after incubation of human hepatocytes exposed to PS-341.

Results:

Compound (induce)	Donor 1		Donor 2	
	CYP 1A2	CYP 3A4	CYP 1A2	CYP 3A4
Rifampin (CYP3A4)		36 fold ↑		2.6 fold ↑
Omeprazole (CYP1A2)	8.9 fold ↑		5 fold↑	
PS-341	2 fold ↑	None	<2-fold	None

Blank box – activity not measured

PS-341 up to 50 μM (concentrations tested - 2.5, 5.0, 10, 25, and 50 μM) caused minimal induction of CYP 1A2 and 3A4 as compared to positive controls.

(7) In Vitro Metabolism of PS-341 by Human CYP Isozymes. Module 4, rpt-00011.

Key study findings: PS-341 was metabolized primarily by the CYP-3A4 and -2D6 isozymes.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc. MA.
Date of study initiation: July 20, 2001
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: PS-341, lot # 5a; purity not mentioned.
[^{14}C]-PS-341, lot # TCH-2591-51-B.

Results:

PS-341 Remaining (%) Following Incubation With Various cDNA Expressed Human CYP Isozymes (n=3).

PS-341 was metabolized in this system primarily by the CYP 3A4 and 2D6 isozymes. Isozymes CYP2D6, 2C19, 1A2, and 2C9 metabolized 50%, 33%, 23%, and 20% of PS-341 at 60 minutes, respectively. Greater than 90% of PS- 341 metabolized by CYP 3A4, 2D6, 2C9, and 1A2 were deboronated PS-341.

(8) Inhibition Potential of PS-341 on CYP1A2, 2C9, 2C19, 2D6, and 3A4 in the Human Microsomal System. Module 4, rpt-00135.

Key study findings: PS-341 is a weak inhibitor of CYP2C19 (IC_{50} ~18 μM).

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.
Date of study initiation: March 7, 2002
Drug, lot #, radiolabel, and % purity: PS-341, lot # 5A. purity not mentioned.

Methods: Human liver microsomes were incubated containing different substrates for CYP isozymes. The metabolisms of these substrates were quantified by LC/MS techniques.

Results:

IC_{50} Values for CYP Isozymes in Human Liver Microsomes are shown below.

	IC_{50} (μM)					
CYP	1A2	2C9	2C19	2D6	3A4	3A4
Substrate	Phenacetin	Tolbutamide	S-mephenytoin	Dextromethorphan	Midazolam	Testosterone
Compound						
PS-341	>30	>30	18	>30	>30	>30
Positive Controls						
Furafylline	3.2 (0.48 ~ 4.4)					
Sulfaphenazole		0.17 (0.5 ~ 0.8)				
Omeprazole			3.3 (4.0 ~ 7.1)			
Quinidine				0.09 (0.02 ~ 0.37)		
Ketoconazole					0.6 (0.04 ~ 0.7)	0.5 (0.04 ~ 0.7)

The numbers in parentheses are the range of IC_{50} values reported in the literature.

PS-341 was not a good inhibitor of CYP isozymes 2C9, 2D6, and 3A4.

(9) Structural Elucidation of PS-341 Metabolites Produced In Vitro by Human Liver Microsomes and cDNA-Expressed Cytochrome P450 Isozymes. Module 4, rpt-00033.

Key study findings: PS-341 was primarily metabolized by CYP 3A4 and CYP 2D6.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., MA.
Drug, lot #, and % purity: PS-341, lot # 5a, purity not mentioned.

Methods: Human liver microsomes (1 mg/ml protein) preparations containing 25 μM PS-341 were incubated at 37^0 C in the presence and absence of NADPH (2 μM). The cDNA-expressed CYP Isozymes incubations were also carried out at 37^{0}C. Proposed structures are based on ES+ MS/MS spectra on isolated fractions.

Results:

Relative Abundance of PS- 341 Metabolites as Percent of Total Metabolism

	M1/M2 (A+B) %	M3 (C) %	M4 (D) %	M5/M6 %	M7 %	M8 %
Microsomes	43	11	4	25	10	7
CYP1A2	85	11	0	<1	4	0
CYP2C9	81	10	0	4	5	0
CYP2C19	58	8	0	10	24	0
CYP2D6[a]	86	6	0	1	7	0
CYP3A4[a]	76	13	0	4	6	0

a: Isozymes primarily responsible for PS-341 metabolism, that is, under controlled incubation conditions, CYP3A4 and CYP2D6 metabolize PS-341 to a greater extent than the other isozymes[2].

Proposed PS-341 In Vitro Biotransformations in Human Liver Microsomes.

(10) Identification and Characterization of PS-341 Metabolites in Humans. Module 4, rpt-00114.

Key study findings: M1, M2 and M4 were the major metabolites detected in human plasma.

Conducting laboratory and location: Millennium Pharmaceuticals, Inc., Cambridge, MA.

Drug, lot #, radiolabel, and % purity: PS-341, lot # 5A and [^{14}C]-PS-341, lot # 2619-117, 68 μCi/mg, purity not mentioned.

Methods: Blood samples from 8 patients in Phase 1 study were collected prior to and at selected time points after they received 1.6-2.0 mg/m^2 PS-341 by iv bolus administration. Samples were analyzed by using methods.

Results:

Estimated Concentration of PS-341 metabolites in Plasma

	10-minute Postdose (pg/mL)	30-minute Postdose (pg/mL)
PS-341[a]	29700	9630
M1	1900	1200
M2	3400	3700
M3	630	470
M4	1500	1700
M5	530	580
M6	740	1000
M7	190	180
M8[b]	110	170
M23	89	73
M24	64	55
M25	ND[c]	ND[c]
M26	ND[c]	ND[c]
M27	< LOD[d]	< LOD[d]
M28	< LOD[d]	< LOD[d]
M29	110	170
M30	430	480

a Average concentration from pharmacokinetic analysis of the samples used in this study (as reported in Study 98-194 pharmacokinetic summary)
b M8 isomers, if present, were unresolved under the SRM chromatographic conditions
c Not detected.
d Intensity less than the limit of detection (signal-to-noise ratio = 3) by visual inspection

Proposed Mechanism for the Degradation of PS- 341 Under Oxidative, Acidic, and Alkaline Conditions is Shown below.

Excretion

(1) **Biliary Excretion of PS-341 in Male Rats Following a Single Intravenous Dose of [^{14}C]-PS-341. Module 4, 6837-114.**

Reviewed under distribution.

(2) **Tissue Distribution, Biliary Excretion, and Mass Balance of ^{14}C-PS-341 in Male Sprague Dawley Rats Following a Single Bolus Intravenous Dose. Module 4, pk-800.**

Reviewed under distribution.

(3) **Biliary Excretion of PS-341 in Cynomolgus Monkeys Following a Single Intravenous Dose of [^{14}C]-PS-341. Module 4, 6837-116 & Module 4, 6837-116 addendum.**

Reviewed under distribution.

(4) **Amended Final Report**
Tissue Distribution and Mass Balance of Total Radioactivity in Male and Female Cynomolgus Monkeys Following a Single Bolus Intravenous Dose of ^{14} C- PS- 341. Module 4, pk-888.

Reviewed under distribution.