Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis

Brendan M. McGuire,[1] Igor A. Zupanets,[2] Mark E. Lowe,[3] Xunjun Xiao,[3] Vasyliy A. Syplyviy,[2] Jon Monteleone,[4] Sharron Gargosky,[5] Klara Dickinson,[5] Antonia Martinez,[5] Masoud Mokhtarani,[5] and Bruce F. Scharschmidt[5]

Phenylbutyric acid (PBA), which is approved for treatment of urea cycle disorders (UCDs) as sodium phenylbutyrate (NaPBA), mediates waste nitrogen excretion via combination of PBA-derived phenylacetic acid with glutamine to form phenylactylglutamine (PAGN) that is excreted in urine. Glycerol phenylbutyrate (GPB), a liquid triglyceride pro-drug of PBA, containing no sodium and having favorable palatability, is being studied for treatment of hepatic encephalopathy (HE). *In vitro* and clinical studies have been performed to assess GPB digestion, safety, and pharmacology in healthy adults and individuals with cirrhosis. GPB hydrolysis was measured *in vitro* by way of pH titration. Twenty-four healthy adults underwent single-dose administration of GPB and NaPBA and eight healthy adults and 24 cirrhotic subjects underwent single-day and multiple-day dosing of GPB, with metabolites measured in blood and urine. Simulations were performed to assess GPB dosing at higher levels. GPB was hydrolyzed by human pancreatic triglyceride lipase, pancreatic lipase-related protein 2, and carboxyl-ester lipase. Clinical safety was satisfactory. Compared with NaPBA, peak metabolite blood levels with GPB occurred later and were lower; urinary PAGN excretion was similar but took longer. Steady state was achieved within 4 days for both NaPBA and GPB; intact GPB was not detected in blood or urine. Cirrhotic subjects converted GPB to PAGN similarly to healthy adults. Simulations suggest that GPB can be administered safely to cirrhotic subjects at levels equivalent to the highest approved NaPBA dose for UCDs. *Conclusion:* GPB exhibits delayed release characteristics, presumably reflecting gradual PBA release by pancreatic lipases, and is well tolerated in adults with cirrhosis, suggesting that further clinical testing for HE is warranted.

(HEPATOLOGY 2010;51:2077-2085)

Abbreviations: AE, adverse event; AUC, area under the concentration versus time curve; CEL, carboxyl ester lipase; C_{max}, maximum plasma concentration; GPB, glycerol phenylbutyrate; HE, hepatic encephalopathy; MELD, model for end-stage liver disease; NaPBA, sodium phenylbutyrate; PAA, phenylacetic acid; PAG, phenylacetylglycine; PAGN, phenylacetylglutamine; PBA, phenylbutyric acid; PBG, phenylbutyrylglycine; PBGN, phenylbutyrylglutamine; PK, pharmacokinetic; PLRP2, pancreatic lipase-related protein 2; PTL, pancreatic triglyceride lipase; UCD, urea cycle disorder.

From the [1]University of Alabama, Birmingham, AL; the [2]National University of Pharmacy and Kharkiv National Medical University, Kharkiv, Ukraine; the Division of [3]Pediatric Gastroenterology, Hepatology, and Nutrition, University of Pittsburgh, Pittsburgh, PA; [4]Pharsight Corp., Cary, NC; and [5]Hyperion Therapeutics, Inc., South San Francisco, CA.

Received October 13, 2009; accepted January 15, 2010.

Clinicaltrials.gov registration numbers: protocol UP 1204-001, ID# NCT00977600; protocol UP 1204-002, ID# NCT00986895. Studies for UP 1204-001 and UP 1204-002 provide assurance that informed consent in writing was obtained from each patient and the study protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the appropriate institutional review committee.

Address reprint requests to: Bruce F. Scharschmidt, M.D., Senior Vice President and Chief Medical Officer, Hyperion Therapeutics, 601 Gateway Boulevard, Suite 200, South San Francisco, CA 94080. E-mail: bruce.scharschmidt@gmail.com; fax: 650-745-3581.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.23589

Potential conflict of interest: K. Dickinson, A. Martinez, M. Mokhtarani, S. Gargosky, and B. F. Scharschmidt are employees of Hyperion or were at the time of the study. None of the other authors have a financial interest in Hyperion, although payments were made by Hyperion to Pharsight Corporation (Jon Monteleone), which performed the pharmacokinetic modeling, to University of Pittsburgh (Mark Lowe) for the studies of in vitro *digestion of glycerol phenylbutyrate, and to the National University of Pharmacy and Kharkiv National Medical University, Kharkiv, Ukraine (Igor Zupanets, V. Syplyviy), where the clinical studies were conducted. Drs. McGuire, Monteleone, and Lowe are consultants for and received grants from Hyperion. Dr. Syplyviy received grants from Hyperion. Drs. Gargosky, Scharschmidt, Martinez, and Dickinson own stocks in Hyperion. Dr. Mokhtarani owns stock in Pfizer.*

Additional Supporting Information may be found in the online version of this article.

Glycerol phenylbutyrate (GPB) [or glyceryl tri-(4-phenylbutyrate), also referred to as HPN-100] is an oral investigational agent under development for hepatic encephalopathy (HE) and urea cycle disorders (UCDs). It is a pro-drug of phenylbutyric acid (PBA), currently marketed as sodium phenylbutyrate (NaPBA), for the treatment of UCDs. It consists of glycerol with three molecules of PBA linked as esters. GPB is a pale yellow, nearly odorless and tasteless oil, whereas NaPBA has palatability issues, high sodium content, and high pill burden. The maximum approved daily dose of NaPBA (20 g) corresponds to 40 tablets containing ≈2,400 mg of sodium, which exceeds the daily allowance of 2,300 mg/day recommended in the US Department of Health and Human Services *Dietary Guidelines for Americans, 2005* for the general population and 1,500 mg/day for individuals with hypertension or sodium retaining states.[1] The corresponding dose of GPB is 17.4 mL, which contains no sodium.

NaPBA mediates excretion of waste nitrogen as shown in Fig. 1. PBA is absorbed from the intestine and converted by way of β-oxidation to the active moiety, phenylacetic acid (PAA). PAA is conjugated with glutamine in the liver and kidney by way of *N*-acyl coenzyme A-L-glutamine *N*-acyltransferase to form phenylacetylglutamine (PAGN).[2] Like urea, PAGN incorporates two waste nitrogens and is excreted in the urine.[3]

Because GPB contains no sodium and may be better tolerated than NaPBA, its safety and pharmacology were studied in healthy adults and adults with cirrhosis, as was the handling of GPB by human pancreatic lipases. Monte Carlo simulations were performed to assess metabolites blood levels and therefore clinical safety at doses approximating the highest approved dose of NaPBA for treatment of UCDs.

Materials and Methods

In Vitro *Hydrolysis of GPB by Pancreatic Enzymes*

Recombinant human pancreatic triglyceride lipase (PTL), pancreatic lipase-related protein 2 (PLRP2), colipase, and carboxyl ester lipase (CEL) were expressed in yeast and purified as described.[4-7] Lipase activity against GPB was measured by titration of the released fatty acid (PBA) at 23°C using a Radiometer TIM 854 pH-stat.[8] The assay buffer contained 0.5 mL (550 mg) of emulsified GPB and 1 mM Tris-HCl (pH 8.0), 2 mM $CaCl_2$, 150 mM NaCl, and 0.5 or 4 mM sodium taurodeoxycholate for PTL and PLRP2 or 10 mM sodium cholate for CEL assays. PTL activity was determined with 3 μg of PTL ± 3 μg of colipase

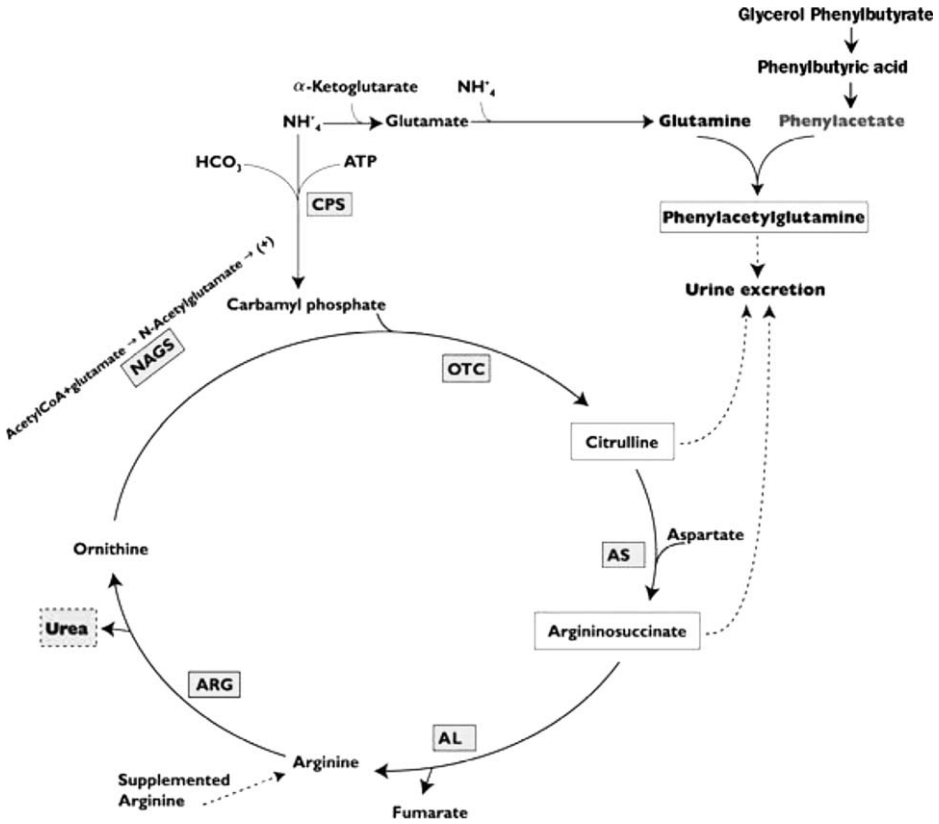

Fig. 1. Urea cycle and removal of waste nitrogen as hippuric acid following administration of sodium benzoate and as phenylacetylglutamine following administration of sodium phenylbutyrate. [Adapted from Summar and Tuchman, J Pediatr 2001;138(Suppl.):S6-S10.]

added at time zero. PLRP2 activity was determined with 10 μg of PLRP2 ± 10 μg of colipase added at time zero. CEL activity was determined with 10 μg of CEL in the absence of colipase. Each reaction was monitored for 5 minutes. The reaction rate was determined from the slope of the linear curve. The rate of 100 mM NaOH titration during the assay was set to maintain a constant pH of 8.0 for PTL and PLRP2 and 50 mM NaOH for CEL. The activity of PTL and PLRP2 against tributyrin and triolein in 1 mM Tris-HCl (pH 8.0), 2 mM $CaCl_2$, 150 mM NaCl, and 4 mM sodium taurodeoxycholate and of CEL against tributyrin and triolein in the same buffer with 10 mM sodium cholate and no taurodeoxycholate was determined using the same methodology.

Study Design and Treatments

UP 1204-001. This was a phase 1, randomized, crossover, open-label study designed to assess safety, tolerability, pharmacokinetic (PK) equivalence, and bioequivalence in healthy adult subjects. Intravenous AMMONUL (a 10%/10% solution of sodium phenylacetate and sodium benzoate) and a formulated oral preparation of GPB were administered in addition to GPB (unformulated) and NaPBA, but only the results for NaPBA and unformulated GPB are reported in this study. Subjects received a single dose of either NaPBA or GPB on separate dosing days, at least 7 days apart. NaPBA and GPB were administered at a dose equivalent to 3 g/m^2 of PBA. PK samples were taken predose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours postdose. Urine was collected from 0-4, 4-8, 8-12, and 12-24 hours postdose. PK variables were calculated for PBA, PAA, phenylacetylglycine (PAG), PAGN, phenylbutyrylglycine (PBG), and phenylbutyrylglutamine (PBGN). A test for intact GPB was also conducted in subjects receiving GPB.

Bioequivalence was assessed by calculating 90% confidence intervals for the ratio of geometric means between test and reference treatments. The ratios and confidence intervals were calculated in an analysis of variance model for log-transformed pharmacokinetic variables including treatment, period, and the treatment by period interaction as fixed effects and subject as a random effect.

UP 1204-002. This was an open-label study of the safety and PK equivalence of GPB in subjects with cirrhosis (Child-Pugh score A, B, or C [n = 8 in each group]) compared with age- and sex-matched healthy subjects with normal hepatic function (n = 8). Subjects received a single oral GPB dose (100 mg/kg/day) on day 1, two doses per day (12 hours apart) on days 8-14 (200 mg/kg/d), and a single dose on day 15 (100 mg/kg/d). The single oral dose on day 1 was a fasting dose, whereas the first dose on day 8 was given with a meal. The last GPB dose was administered on the morning of day 15 and was followed by 48 hours of plasma PK sampling and urine collection. PK blood samples were drawn at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours postdose on days 1, 8, and 15, and at 48 hours after dosing on days 1 and 15. Urine was collected from 0-4, 4-8, 8-12, and 12-24 hours postdose on days 1, 8, and 15 and at 24-48 hours postdose on days 1 and 15. PK samples were drawn fasting prior to the morning dose (trough) and 2 hours postdose on days 9-14. A 12-lead electrocardiogram was performed at screening on days 0 and 7, 2 hours postdose on days 1 and 15 (between 9:00 AM and 10:00 AM), and at follow-up (7 days after day 15).

Pharmacokinetic Analyses

Plasma and urine PK parameters were calculated for all subjects and summarized with descriptive statistics (number of patients, mean, standard deviation, median, minimum, and maximum). PK parameters were calculated using time concentration profiles for each subject, including area under the concentration versus time curve from time 0 (predose) to 24 hours (AUC_{0-24}), calculated using the linear trapezoidal rule; maximum plasma concentration at steady state (C_{max}); and the time of maximum plasma concentration at steady state. The amount of PAGN excreted in urine over 24 hours was calculated from urinary concentration (by multiplying the urinary volume with urinary concentrations).

Pharmacokinetic Modeling/Dosing Simulations

Monte Carlo simulations were performed to predict the average and uncertainty (5% and 95% prediction intervals) for simulated plasma PBA, PAA, and PAGN concentrations in a hypothetical clinical trial with 5,000 cirrhotic subjects dosed with GPB at 9 mL (≈9.9 g) twice daily. A concentration time profile was developed for each analyte corresponding to the mean as well as the 5% of patients with the highest and lowest levels.

The population PK model and corresponding PK parameter estimates used for the Monte Carlo simulations were developed using Nonmem VI (NONMEM; ICON Development Solutions, Ellicott City, MD) and PK data from protocols UP 1204-001 and UP 1204-002 and a phase 2 study in UCD patients (protocol UP 1204-003).[9] Simulations were preformed

Table 1. Activity of Pancreatic Lipases Against GPB

	Lipase (U/mg)	
	With Colipase	Without Colipase
0.5 mM sodium taurodeoxycholate		
PTL (3 μg)	618	342
PLRP2 (20 μg)	35	32
4 mM sodium taurodeoxycholate		
PTL (3 μg)	592	42
PLRP2 (20 μg)	22	11
10 mM sodium cholate		
CEL (10 μg/mL)	249	

Values are the average of 2 to 3 determinations and are expressed as μmole FA released/minute/mg protein or U/mg.

using Trial Simulator software (TS2; Pharsight Corporation Inc., Mountain View, CA), assuming dosing at 8:00 AM and 6:00 PM (to coincide with breakfast and dinner), 7 days of dosing to ensure steady state concentrations were achieved, and frequent sampling (daily samples at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24 hours). Because body surface area was a significant demographic covariate for clearance and volume of distribution parameters in the PK model, simulation was used to generate this demographic variable for each of the 5,000 hypothetical patients.

Results

In Vitro *Hydrolysis of GPB by Pancreatic Enzymes*

PTL, PLRP2, and CEL all hydrolyzed GPB (Table 1). The specific activity (μmole fatty acid released/min/mg protein or U/mg) of PTL (≈600 U/mg) was ≈27-fold higher than that of PLRP2 (≈22 U/mg) when both were assayed in the presence of colipase and 4 mM sodium taurodeoxycholate and ≈2.4-fold higher than that of CEL (≈250 U/mg). For comparison, under the same assay conditions, the activity against tributyrin was 4,600 ± 30 U/mg for PTL, 200 ± 9.0 U/mg for PLRP2, and 260 ± 12.0 U/mg for CEL and against triolein was 1,600 ± 153 U/mg for PTL, 120 ± 40 for PLRP2, and 30.3 ± 6.0 for CEL.

Patient Demographics and Disposition

Twenty-four healthy adults were enrolled in protocol UP 1204-001, of whom 22 received each of the study drugs and completed the study according to the protocol.

Thirty-two subjects met the entry criteria and enrolled in protocol UP 1204-002 (Supporting Table 1). All subjects completed the study and were included in the analyses. Subject groups were generally well matched. There were more men than women in Child-Pugh groups A and B and equal numbers of men and women in Child-Pugh C and the healthy volunteer groups. None of the subjects in Child-Pugh A or the healthy volunteer group had HE or ascites. All subjects in Child-Pugh group B had mild ascites and stage I HE, and all subjects in Child-Pugh group C had mild or moderate ascites and stage I or II HE. Serum albumin, creatinine, and international normalized ratio were similar in all subject groups. Serum bilirubin increased with the degree of hepatic impairment, ranging from 0.74 mg/dL in the healthy volunteers to 3.46 mg/dL in Child-Pugh group C. Mean (standard deviation) Child-Pugh and model for end-stage liver disease (MELD) scores, respectively, increased commensurate with Child-Pugh grade (A = 5.8 [0.5] and 7.3 [1.3]; B = 8.3 [0.5] and 8.6 [2.1]; C = 10.6 [0.5] and 12.6 [2.8]) among the cirrhotic subjects, and all 32 subjects had negative drug screens and alcohol breath test results at all assessments.

Safety and Tolerability (Supporting Table 2)

Protocol UP 1204-001. Twenty-one adverse events (AEs) were reported by 10 subjects while receiving NaPBA compared with six AEs by two subjects while receiving GPB. The most frequently reported AEs with NaPBA were dizziness (n = 5), headache (n = 4), and nausea (n = 3). One patient reported epigastric discomfort and one patient reported vomiting (n = 2) while taking GPB.

Protocol UP 1204-002. There were no SAEs or AEs leading to withdrawal during the study. Overall, AEs were reported in 26 of 32 subjects. Among healthy volunteers, five of eight reported AEs, whereas seven of eight subjects in each of the Child-Pugh groups reported AEs. The most common system organ class was investigations (18 subjects); increased body temperature was reported by 10 subjects with cirrhosis and decreased platelet count was recorded for four subjects in Child-Pugh group A and one subject in healthy volunteer group D. Other common classes of AEs included gastrointestinal complaints (n = 11) and nervous system disorders (n = 8), particularly headache (n = 7). Most AEs were considered not related (n = 9) or possibly related (n = 20) to the study medication, and no AEs were considered definitely related. Analysis of vital signs including oral temperature did not reveal clinically or statistically significant changes from baseline. The highest mean temperature recorded in any treatment group at any time was 37.2°C, and the highest temperature recorded in any

Table 2. Plasma Pharmacokinetics of PBA, PAA, and PAGN in Clinical Studies UP 1204-001 and UP 1204-002

Analyte	Treatment	C_{max} (μg/mL)	T_{max} (hours)	AUC_t (μg·h/mL)
Study UP 1204-001: healthy volunteers (single dose = 3 g/m^2/day PBA mole equivalent)				
PBA	NaPBA	221.0 (44.0)	0.9 (0.61)	538.2 (111.6)
	GPB	37.0 (21.74)	2.4 (0.8)	133.5 (74.7)
PAA	NaPBA	58.8 (10.37)	3.9 (0.3)	266.6 (57.4)
	GPB	14.9 (6.86)	4.0 (1.0)	70.90 (36.04)
PAGN	NaPBA	63.1 (7.14)	3.2 (0.4)	379.9 (59.4)
	GPB	30.1 (8.95)	4.0 (0.8)	278.1 (99.1)
Study UP 1204-002: healthy volunteers and cirrhotic patients (dose = 100 mg/kg twice daily)*				
PBA	Child-Pugh A	42.81 (25.53)	2.25 (0.65)	131.65 (74.06)
	Child-Pugh B	41.83 (26.22)	2.88 (0.95)	189.51 (141.55)
	Child-Pugh C	44.33 (21.50)	3.13 (1.55)	192.08 (82.90)
	Volunteers	29.80 (14.15)	3.00 (0.76)	132.68 (45.24)
PAA	Child-Pugh A	33.15 (14.66)	3.75 (1.16)	168.80 (155.76)
	Child-Pugh B	30.85 (19.82)	4.50 (1.69)	252.35 (299.13)
	Child-Pugh C†	53.08 (64.49)	4.75 (2.38)	579.85 (1150.54)
	Volunteers	25.52 (16.05)	3.63 (0.52)	130.48 (121.45)
PAGN	Child-Pugh A	37.67 (9.33)	3.88 (0.99)	335.10 (145.63)
	Child-Pugh B	38.10 (15.20)	4.00 (1.69)	466.89 (359.11)
	Child-Pugh C	43.09 (15.27)	5.25 (2.05)	578.41 (540.32)
	Volunteers	46.27 (15.07)	4.25 (0.71)	550.89 (171.53)

Values are expressed as the mean (standard deviation).

Abbreviations: AUC_t, area under the plasma concentration curve from time 0 to the last measurable plasma concentration C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration.

*Protocol UP 1204-002 involved administration of glycerol phenylbutyrate (GPB) only and did not include a sodium phenylbutyrate (NaPBA) comparator arm. AUC values represent the AUC from time 0 to the last measurable plasma concentration. (AUCt [(μg/mL)/hour]).

†The mean PAA C_{max} and AUC_t are considerably higher than other groups in group C due to one subject who exhibited unusually high level of PAA.

individual subject at any time point was 38.2°C. Abnormal laboratory safety findings were common in subjects with hepatic impairment. There was no consistently observed pattern among hematology, coagulation, or chemistry (including liver enzymes), and changes after 7 days of dosing with GPB were clinically insignificant. Clinically significant changes in electrocardiogram were not observed with GPB dosing, nor were changes observed in the QTc intervals.

Pharmacokinetic Analyses

Protocol UP 1204-001. NaPBA resulted in higher plasma levels (both C_{max} and AUC) of PBA, PAA, and PAGN than GPB; the 90% confidence intervals for the ratio of geometric means of each metabolite following GPB compared with NaPBA extended below the commonly used lower bioequivalence level of 0.8 (Fig. 2 and Table 2). The mean plasma half-lives of PBA, PAA, and PAGN were 0.7 (± 0.1) hours, 1.2 (± 0.2) hours, and 1.7 (± 0.5) hours, respectively, after NaPBA administration. The mean plasma half-life of PBA after GPB administration was 1.9 (± 1.7) hours and ranged from 0.8 to 7.4 hours. The plasma half-lives for PAA and PAGN after GPB administration ranged from 1.0 to 1.8 hours and 1.9 to 16.9 hours, respectively.

Urinary excretion of PAGN was higher following NaPBA than following GPB (Table 3). However, urinary collection of PAGN was incomplete at 24 hours following GPB dosing, as PAGN was still detectable in plasma at 24 hours. In contrast, PAGN following NaPBA dosing was undetectable in the plasma by 24 hours and PAGN elimination considered complete by 24 hours. Taking into account the pattern of urinary excretion and plasma levels following the single dose arm of study UP 1204-002, the 0-48 hours urine collection was split into 0-24 and 24-48 hours to calculate the percentage of urinary PAGN that occurred after 24 hours (Table 4). It is estimated that ≈15% of urinary PAGN excretion in patients on GPB occurred beyond 24 hours, the time the collection was terminated for study UP 1204-001. When corrected for the

Table 3. Urinary Output of PAGN in Study UP 1204-001 (Healthy Volunteers)

	NaPBA	HPN-100	HPN-100c*
PAGN (0-24 hours) amount excreted	4,905.0 (1414)	4,130.3 (925)	4,749.9

All values are expressed as the mean (standard deviation). Single dose: 3 g/m^2/day PBA mole equivalent.

*HPN-100c value is corrected for approximately 15% under collection of urinary PAGN. PAGN was detectable in plasma samples of subjects receiving GPB but not NaPBA after the 24-hour time point, indicating that urinary collection of PAGN was incomplete at 24 hours following GPB dosing.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.