Study title: Influence of Time and Temperature on the Metabolism of GPI 15715 by Alkaline Phosphatase

Study number: Metab\dmpk-06-083

Study design: To evaluate the role of alkaline phosphatase in the metabolism of GPI 15715, the Sponsor conducted two experiments. In the first experiment, the time course of the GPI 15715 metabolism at different concentrations (1, 10, and 50 μM) was assessed in vitro over a time period of 30 mins. In the second experiment, the stoichiometry of GPI 15715 (2.5 μM) and its metabolites were analyzed at different temperatures ranging between 37-28 °C.

Results and Comments: GPI 15715 was rapidly metabolized by alkaline phosphatase, 2/3rd of the substrate metabolized within 5- 10 mins, the metabolism was complete within 30 mins. The rate of metabolism was approximately constant and independent of the initial substrate concentration. The metabolism rate decreased with decrease in the temperature. Similarly the production of formaldehyde and propofol decreased with decrease in temperature. No further metabolism of GPI 15715 occurred in the presence of alkaline phosphatase.

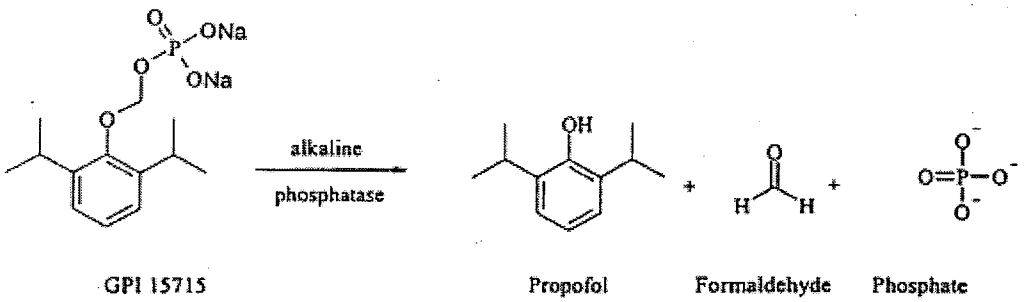

Figure A: Metabolism of GPI 15715 by alkaline phosphatase

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Figure 2: Reaction Time VS. Percent GPI 15715 Metabolized (Study #1)

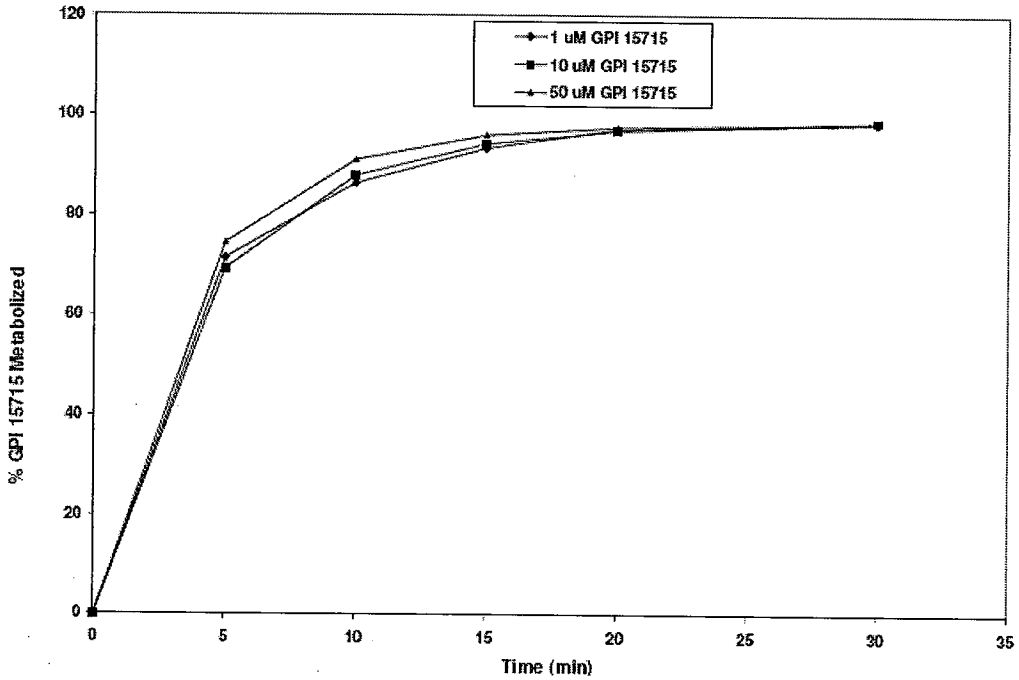


Figure 3: Metabolism of GPI 15715 by Alkaline Phosphatase at Various Incubation Temperatures (Study #2)

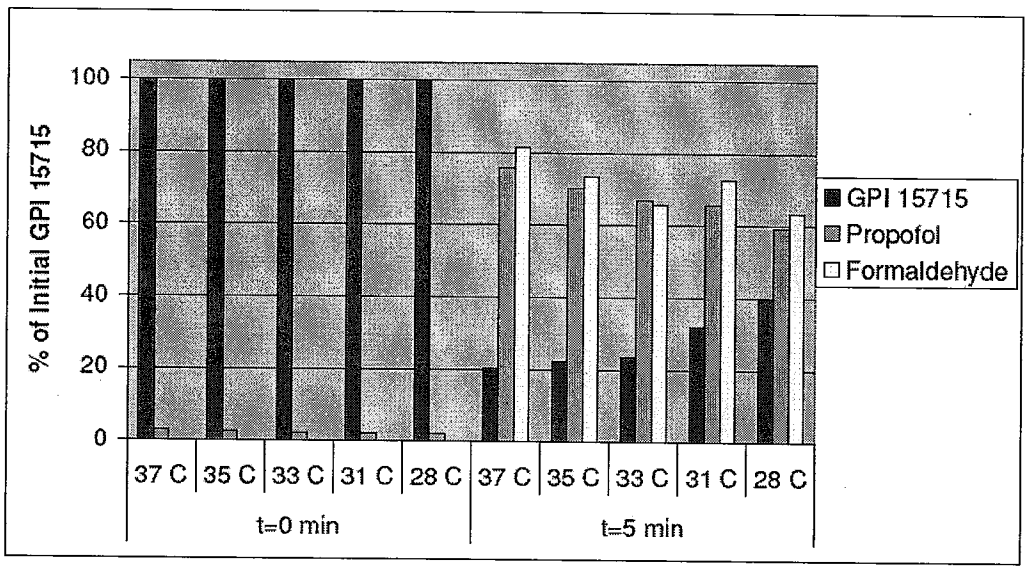
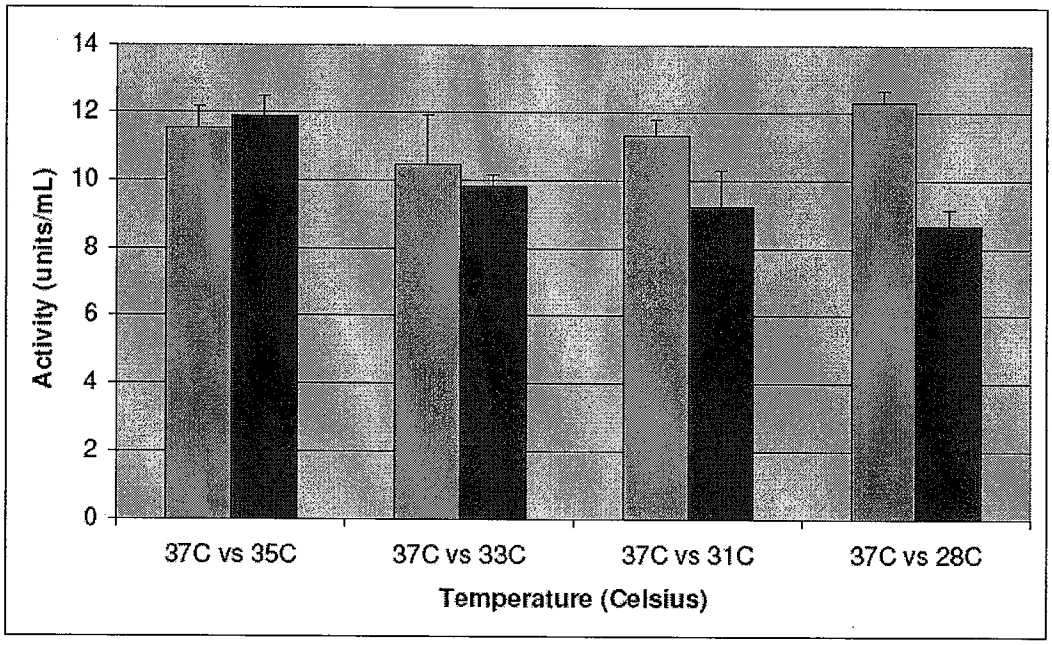


Figure 4: Alkaline Phosphatase Activity at Various Incubation Temperatures Measured by [redacted] (Study #2) b(4)



2.6.4.6 Excretion

Study title: Pharmacokinetics and Mass Balance of [^{14}C] GPI 15715 Following Intravenous Administration to Sprague-Dawley Rats

Study number: Excr\dm-00-007-snaz-102

Study design: ^{14}C GPI 15715 was administered intravenously in Sprague Dawley rats (4/sex/group) at a target dose level of 18.65 mg/kg. Urine, feces, and cage rinses were collected at different time points up to 168 hrs post dosing. The study was conducted in the [redacted] under GLP conditions. b(4)

Results and Reviewer's Comments: Urinary excretion of the radioactive GPI 15715 in males and females was found to be approximately 66 and 76% respectively indicating urine as the major route of excretion. Fecal excretion was observed to be a minor route representing approximately 22 and 10% elimination. The caracass retained ~2% of the radioactivity and the cage rinses and wipes account for the rest of the analyzed radioactivity. The mean total recovery of the radioactive compounds was 91% in males and 90% in females.

Table 18. Excretion after a Single Dose of ^{14}C-Fospropofol Intravenously to Rat (DM00-007)				
Sex	Dose (mg/kg)	Mean Percentage of Administered Dose (SD)		
		Urine	Feces	Total[a]
Male	18.64	65.7% (4.25)	21.9% (2.37)	91.1% (1.91)
Female	18.64	76.3% (9.06)	10.4% (0.960)	90.5% (9.84)

SD= Standard deviation
[a]Includes cage wash and rinses and carcass amounts

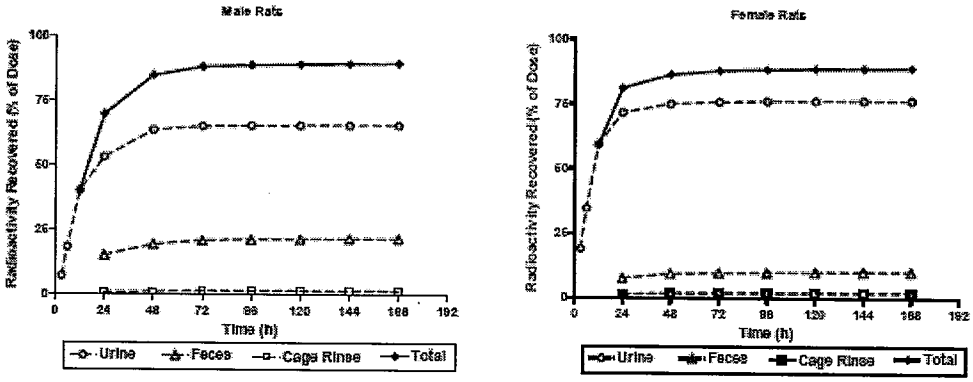


Figure 2. Cumulative Amount of Radioactive Material Recovered after a Single Intravenous Dose of ^{14}C- Fospropofol to Male and Female Rats (DM-00-007)

Study title: Absorption, Excretion, and Pharmacokinetics of [^{14}C] GPI 15715 Following Intravenous Administration to Beagle Dogs

Study number: Excr\dm-00-007-snaz-103

Study design: ^{14}C GPI 15715 was administered intravenously in beagle dogs (3/sex/group) at a target dose level of 18.65 mg/kg. Urine, feces, plasma, and cage rinses were collected at different time points up to 168 hrs post dosing. The study was conducted in the [redacted] under GLP condition. b(4)

Results and Reviewer's Comments: Urinary excretion of the radioactive GPI 15715 in male and female dog was found to be approximately 65 and 77% respectively indicating urine as the major route of excretion. Fecal excretion was observed to be a minor route representing approximately 15 and 10% elimination. The cage debris rinses and wipes account for 4-6% of the radioactivity. The mean total recovery of the radioactive compounds was 85% in males and 91% in females. The rate of elimination was found to be faster in females in the first 12 hrs; however, the excretion was nearly complete in males and females by 48 hrs.

The blood concentration in males and females were 47-54 µg equivalents and was observed to decrease overtime as indicated in the Sponsors' table 4.

Table 19 Excretion after a Single Intravenous Doses of ^{14}C- Fospropofol Intravenously to Dog (DM00-008)

Sex	Dose (mg/kg)	Mean Percentage of Administered Dose % (SD)		
		Urine	Feces	Total[a]
Male	18.64	64.6% (4.58)	14.9% (1.20)	85.8% (2.63)
Female	18.64	76.6% (7.20)	10.0% (1.37)	91.0% (2.71)

SD= Standard deviation
[a]Includes cage wash and rinses

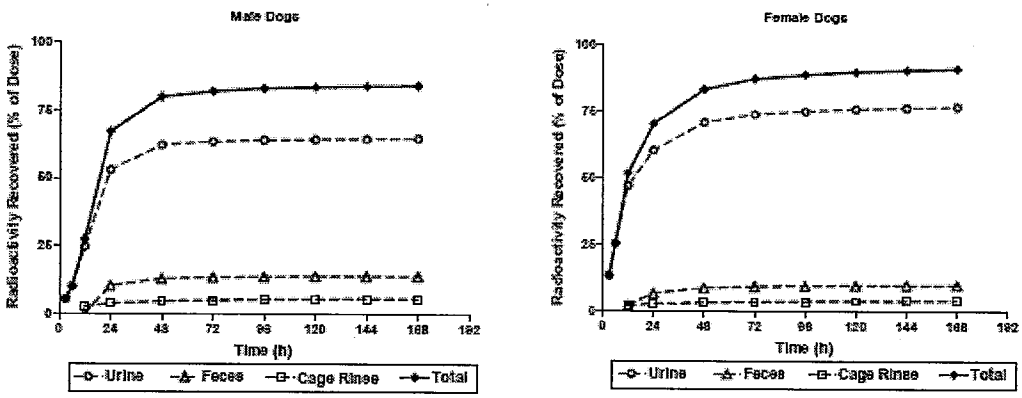


Figure 4. Cumulative Amount of Radioactive Material Recovered after a Single Intravenous Dose of ^{14}C- Fospropofol to Male and Female Dogs (DM00-008)

2.6.4.7 Pharmacokinetic drug interactions

There were no nonclinical pharmacodynamics drug interaction studies conducted for this application.

2.6.4.8 Other Pharmacokinetic Studies

There were no nonclinical pharmacodynamics drug interaction studies conducted for this application.

2.6.4.9 Discussion and Conclusions

GPI 15715 has been adequately examined in vitro and in vivo for understanding its ADME profile. GPI 15715 was metabolized rapidly to form propofol, phosphate, and

formic acid in the presence of alkaline phosphatase in nonclinical species such as rat, dog, and monkey. GPI 15715 is metabolized in human also by alkaline phosphatase, indicating that the choice of rat, dog and monkey for toxicology studies was appropriate. Alkaline phosphatase is known to be widely distributed in the tissues; therefore complete conversion of the propofol in vivo is expected. The formaldehyde is also found to have undergone enzymatic digestion rapidly via formaldehyde dehydrogenase (FDH). Formic acid is also a byproduct of the Kreb cycle and the enzyme metabolizing formic acid to formate is present in appreciable amount in all tissues and it is expected that formic acid formed after GPI 15715 is metabolized rapidly to formate. In the toxicokinetic studies formate level after the GPI 15715 administrations was observed to be similar to the background level. The phosphate levels were also assessed in the toxicology studies and were found to remain unchanged after GPI 15715 administrations.

In vitro and in vivo studies indicate CYP 450 is not a substrate of GPI 15715 and does not play any role in the metabolism of GPI 15715, therefore, drug-drug interactions with GPI 15715 is anticipated to be minimal. The test article is highly protein bound. It does not interact with its metabolites indicating that their interactions with highly protein bound drugs are unlikely.

The AUC and Cmax of after the IV administration GPI 15715 increased dose proportionally in all of the species studied. The in vivo excretion and tissue distribution studies were conducted after IV administration of GPI 15715 since IV is clinical route of administration. The elimination half life of GPI 15715 was observed to be short; therefore no systemic accumulation is expected. There was a difference in the elimination pattern in rat which is biexponential compared to the other species studies such as dogs. In a mass balance study in rats and dogs 91 and 88% elimination of the GPI 15715 related radioactivity was noted to be via urine and feces, similar route of elimination are expected in human. GPI 15715 was observed to be distributed immediately following its administration in the adrenal gland, liver, kidney, bone marrow, salivary gland, thyroid, and lungs with a concentration of 81.2, 72.2, 47.5, 45.4, 34.2, 24.9, and 19.8 µg respectively. The exposure in the intestine was noted approximately between 3-8 hrs suggesting biliary excretion as a major pathway for elimination of the test article. The highest tissue: plasma ratios in fat (8 hrs), liver (8hrs), kidney (120 hrs), and muscles (120 hrs) were observed to be 5.67, 4.58, 4.0, and 2.73 µg respectively. At 5-days post dosing, the GPI concentration in the tissues with tissue:plasma ratios greater than one were kidney, liver, muscle, lung, large intestine, pigmented skin, bone, small intestine, salivary glands, and thymus with ratios 4.0, 3.3, 2.7, 2.5, 1.8, 1.6, 1.5, 1.4, 1.1, and 1.0 respectively. The in vitro blood partitioning assay showed minimal partitioning of GPI 15715 into the blood cells in rat and dog, and an increased partitioning of GPI 15715 into the blood cells in the rabbits and monkey indicating a species difference.

In summary, the ADME profile of GPI 15715 is observed to be similar to the nonclinical species studied as regards to its absorption, metabolism, and elimination profile. The tissue distribution of GPI 15715 is expected to be similar in primates considering similar protein biding and blood partitioning profile. Wider distribution of GPI 15715 in tissues

is expected in human compared to the rodents because increased blood partitioning in primates compared to that of the rodents were noted.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The Sponsor conducted single dose toxicity studies in Sprague Dawley rats, CD-1 mice, beagle dog, and cynomolgus monkeys with GPI 15715 to determine the safety and efficacy of the induction and maintenance of anesthesia.

In rats clinical signs of anesthesia were observed at a single intravenous bolus dose of 80 mg/kg, the dose > 80 mg/kg showed lethality in these rats. The CD-1 mice did not show any mortality < 160 mg/kg; however, no clinical sign of anesthesia were noted in these animals indicating that the mice were less sensitive to GPI 15715 treatments.

In the beagle dog (n=3, males only) GPI 15715 was administered for the induction of anesthesia with an intravenous bolus administration of 38 mg/kg, the animals were then maintained for 6 hrs with an intravenous infusion dose of 70, 80, or 90 mg/kg/hr for 6 hrs. The animals were sacrificed between 2-3 days post loading dose. Clinical signs of anesthesia were noted in all animals. There was no mortality in this study; however, one animal from the high dose group was dropped from the study due to a drop in its blood pressure. The decrease in heart rate and blood pressure were noted in all treated animals. A prolongation in QTc interval was noted > 20 secs in all low, mid and high dose animals up to 6 hrs post loading dose which was recovered by 50 hrs post loading.

There were two single dose toxicity studies conducted in the cynomolgus monkeys. From the first study the optimal dose for GPI 15715 for the induction and maintenance of anesthesia (1/sex/group) was obtained. GPI 15715 at an intravenous induction dose of 45.5 mg/kg and infusion of up to 64 mg/kg/hr for 8 hrs were observed to be well tolerated in monkeys. The second single dose toxicity in the monkey was conducted to obtain the maximum tolerated dose, the doses used were 38, 44, 50, and 56 mg/kg, same animals (n=2/sex/group) were used in the study for different doses. GPI 15715 induced anesthesia within 2-4 mins in all animals at all doses as evaluated by the assessment of reflexes. All animals showed a decrease in the heart rate and MAP after GPI 15715 administration. The toxicokinetic analyses of the formate produced after the GPI 15715 and formaldehyde administration in the second study in the monkey showed that the

Cmax of formate after formaldehyde administration was higher. However, the overall systemic exposure as indicated by the AUC was similar in the animals administered either with formaldehyde or GPI 15715.

The single dose studies in rat, dog, and monkey did not include adequate number of animals and sometime both genders were not used, however, although not ideal, single dose studies with GPI 15715 provided acute toxicity information for the single dose clinical trial. Because, the single dose studies were done either to find an appropriate dose or to reach the maximum tolerated bolus dose for intravenous administration of GPI 15715, the study designs did not always mimic the clinical protocol, however, provided the total daily intake and adverse effect related to it. Pilot studies with a combination bolus and infusion intravenous administration of GPI 15715 was conducted in rats, dogs (only in males), and monkeys (inadequate number of animals). In rats, the study procedure was not well tolerated. In dogs after 6 hrs of continuous infusion an increase in the QTc prolongation time was noted in all dosages studies (low dose in dog = 458 mg/kg, HED =254 mg/kg) which is, however, recovered by 50 hrs. Note that no such changes were observed in a continuous infusion study with > 24 hrs of infusion with higher dose in the presence of ventilation, therefore QTc prolongation phenomena was believed to be due to the lack of ventilation. In monkeys, 8 hrs of continuous infusion of GPI 15715 was well tolerated (557 mg/kg, HED=180 mg/kg) only adverse events noted were decreased heart rate and arterial pressure. The plasma formate concentrations were observed to be comparable after a single bolus administration of 50 and 20 mg/kg of GPI 15715 and formaldehyde respectively.

The Sponsor conducted repeat dose toxicity studies in Sprague Dawley rats, beagle dogs, and cynomolgus monkeys to determine the safety and efficacy of the induction and maintenance of anesthesia.

The repeat dose study in the Sprague Dawley rats (5/sex/group) was conducted by the continuous intravenous infusion of a single dose of GPI 15715 (47.5 mg/kg) for 1, 2, and 4 hrs for 14 consecutive days, therefore the total daily exposures were 47.5, 95, 190 mg/kg/day. Note that the study design does not mimic the clinical scenario where a combination of intravenous bolus and infusion is used. In this study, a separate group of animals were treated with propofol 20 mg/kg/hr for 4 hr/day for 14 consecutive days. The toxicokinetic analysis of GPI 15715 derived propofol showed extensive variability; the $AUC_{0\text{-}4}$ at low, mid, and high dose ranged to 9-109, 4-24, and 19-84 µg.h/mL. There was no apparent accumulation of propofol following the repeat dose administration in rat. No gender differences were noted. The elimination half life ranged between 0.3-1.5 hrs indicating rapid elimination. The histopathological lesions associated with chronic inflammation of lungs, acute inflammation in liver, cardiomyopathy, bone marrow cell hyperplasia in femur, extramedullary hematopoesis in spleen, and congestion in kidney. The incidence of lung lesions were higher in the test article treated animals than the controls, however, not dose related and was described to be associated with infiltration of foreign particles such as hair and skin structures. Increased incidence of cardiomyopathy in the heart was noted in GPI 15715 treated animals with 2 hrs and 4 hrs of continuous infusion/day compared to those of the controls. The severity index was minimal in all

animals except 2 females with 2 hrs infusion regimen where the severity was described as moderate. There was an increased incidence of acute inflammation in liver characterized as minimal to mild in severity in the test article treated animals compared to those of the controls. There was also an increased incidence of congestion in the kidney; no such changes were noted in the control animals. The changes in the kidney, liver, bone marrow cell hyperplasia, and extramedullary hematopoesis in spleen was also noted in the propofol treated animals. However, incidences of lesions in the injection sites in the test article treated animals but not in propofol treated animals were noted at mid and high doses. The lesions were described as chronic active inflammation characterized by severe in nature in most animals. The lesions consisted of polymorphonuclear cell infiltration in the fibrin strands; the surrounding fibrovascular area was infiltrated with macrophages and multinucleated giant cells. Several cases had a focal area of hemorrhage and were diagnosed as hematoma. The injection site findings were found in much lower frequency in the control and the propofol treated animal. NOAEL for this study was determined to be < 47.5 mg/kg (HED = 7.6 mg/kg) because of the higher incidence of histopathological lesions in liver and lungs compared to those of the control animals.

The were two types of the repeat dose toxicity study in dogs namely continuous infusion for a long period of time ≥ 24 hrs and a repeat dose toxicity study for 14 consecutive days with a bolus and infusion of 1 hr/day with the test article. In the continuous infusion study, the beagle dogs (1/sex/group) were induced to anesthesia by bolus (8-16 mg/kg) intravenous administration of GPI 15715. The animals were then maintained in anesthesia via continuous infusion (68-79 mg/kg/hr). The male dog died at approximately 29 hr post loading dose and the female dog died during recovery after 24 hr of continuous infusion. The continuous infusion of GPI 15715 (TDI female: 1640 mg/kg, HED=911 mg/kg, male: 1888 mg/kg, HED=1048 mg/kg) were not tolerated in dogs. The toxicokinetic analysis of propofol derived from GPI 15715 showed that the Cmax in the male and the female dogs were approximately 30 and 15 µg/mL respectively and was achieved at 1 min post dosing. The heart rate and MAP decreased in both animals. There was a decrease in RBC, hemoglobin, and hematocrit in both of the animals. The histopathology findings include mainly lesions in lung, stomach, liver, injection site, and kidney. The changes in the liver consisted of severe glycogen depletion in both the male and the female. The kidney lesion in both dogs consisted of focal mineral deposits in the medulla (minimal). There was also an injection site reaction near the insertion area of the catheters. In addition, the lungs of both dogs had congestion, edema, hemorrhage, and interstitial cell inflammation with infiltration of alveolar macrophages. In the lung from the male amorphous eosinophilic material (moderate) was also observed in the intraluminal space in the bronchi. The changes in the stomach was observed only in the male and consisted of brown pigmented material (moderate), edema, hemorrhage, congestion, venous thrombi, and necrosis. The histopathological lesion was observed in the trachea (slight-severe) of the female only and consisted of edema, hemorrhage, ulcers, and acute/subacute inflammation involving mucosa and muscularis. The Sponsor mentioned that the histopathological changes such as injection site and tracheal lesions were related to trauma associated with the catheter insertion procedure. The changes observed in the kidney were within the historical

control range of the laboratory conducting the study. The changes in the lung, liver, and stomach were related to the anesthetic effect of the test article according to the Sponsor. Propofol was not used as a comparator in this study. The reviewer can not confirm the Sponsor's conclusion of the findings because there was no experimental control in the study.

In the 14 day repeat dose toxicity study in dogs, (3/sex/group) were administered with the induction dose (24-38 mg/kg) of the test or the positive control (10 mg/kg) by intravenous bolus injection for 30-90 seconds followed by the intravenous infusion (~65-95 mg/kg/hr/day for GPI 15715 and 34-41 mg/kg/hr/day) approximately one hour per day for fourteen days. There was a comparable decrease in the heart rate and MAP in all GPI 15715 and propofol treated animals. There was a comparable decrease in the RBC, hemoglobin, and hematocrit in all GPI 15715 and propofol treated animals. There was evidence of respiratory acidosis in arterial blood samples collected during the final infusion interval with propofol and GPI 15715, the changes in the blood gas analyses indicate that there was depression in the respiratory centers resulting in insufficient alveolar ventilation and CO_2 accumulation. The histological findings are restricted mainly to lung, injection site, skin, bone marrow hyperplasia, and trachea indicating that these are the major target organs for toxicity. The lesion in lungs was associated with increased incidence of the chronic active inflammation of the visceral pleural, and interstitium of lungs after GPI 15715 administration. Similar findings were noted after propofol administration in the interstitium of lungs but not in the visceral pleural area. Another major finding is the metaplasia in the squamous area of trachea. The incidence and the degree of severity increased slightly in the GPI 15715 and propofol treated animals. The Sponsor did not provide peer review of the histological findings, therefore the nature of the lesions are unknown. Trauma associated with the manipulation of the catheter in 3 dogs in the GPI 15751 treated animals exacerbated the severity of the findings. According to the reviewer GPI 15751 (TDI = 133 mg/kg, $AUC_{0\text{-infinity}}$ = 24 μg.h/mL, HED =74 mg/kg) induced an increase in the incidence of the histological findings and/or increase in the severity of the findings in the lungs and injection site compared to those of the propofol (TDI = 51 mg/kg, $AUC_{0\text{-infinity}}$ = 20 μg.h/mL, HED = 74 mg/kg).
There were two different types of the repeat dose toxicity studies conducted in monkeys with GPI 15715. These studies are a continuous infusion for a long period of time ≥24 hrs and a repeat dose toxicity study for one month in which a bolus and infusion of 3 hr/day, 3x/week of the test article was administered.

In the continuous infusion study, the cynomolgus monkeys (3/sex/group) were induced to anesthesia by bolus (20 mg/kg) intravenous administration of either GPI 15715 or propofol (10 mg/kg). The animals were then maintained in anesthesia via continuous infusion of either GPI 15715 (57 mg/kg/hr) or propofol (30 mg/kg/hr). There were 4 unscheduled deaths in this study. The propofol infusion in males resulted in 2/3 (animals #s 1265 and 1266) deaths between 36-42 hrs. Two animals (one male and one female) also died after GPI 15751 administrations. One male (animal #2266) died during recovery at 48 hrs of the study drug administration) and one female (animal # 2767 died at 22.8 hrs). The death in males appeared to be related to the duration of infusion and/or

the total dose received. Each of these animals showed ECG abnormalities, cardiac myodegeneration, edema, and hemorrhage. The female was sacrificed due to humane reason related to the severe subcutaneous edema. The toxicokinetic analyses were designed to compare the exposures of propofol after the IV administration of propofol itself with the prodrug (GPI 15715). The exposures of propofol as described by AUC of were comparable in all animals (230-330 μg.h/mL). The Cmax was higher in the propofol (23 μg/mL) treated animals compared to that of the GPI 15715 (18 μg/mL) treated animals. The formate exposures were similar in all groups (2437-2680 μg.h/mL). There were evidence of blood loss in 3/6 animals from the propofol treated group. Similar incidence of blood loss was noted in the GPI 15715 treated animals. Two females from the propofol group vomited blood. In two females from the GPI 15715 treated group blood was noted in the endotracheal tube. One male from the GPI 15715 treated group had a black tarry stool. In addition, edema, swollen eyelids, swollen tongues were seen occasionally in both propofol and GPI 15715 treated animal. There was a decrease in heart rate and blood pressure in all animals treated with GPI 15715 and propofol and a consistent decrease in the hematocrit level during infusion which was not recovered at the time of necropsy in all animals treated with GPI 15715 and propofol. A decrease in pH values apparently related to an accumulation of CO_2 and a compensatory increase in HCO_3. The CO_2 accumulation was attributed to insufficient alveolar ventilation as a result of the depression of the respiratory centers in the brain by the anesthetics. The increase in pH indicates an acidotic condition in the animals. Similar changes were not always observed in the GPI 15715 treated animals. The histological findings were restricted mainly to striated muscles such as heart and skeletal muscle. There were test article related histopathological changes also in the spleen and the skin. Histological lesion in heart consisted of atrial and left ventricular subendocardial myocardial degeneration accompanied by neutrophilic infiltrates corroborating cardiac insufficiency. Some animals surviving to scheduled necropsy had increased neutrophilic infiltrates with myocardiocytes having large nuclei (karyomegaly) with prominent nucleoli. The changes in the skeletal muscles were associated with fibrovascular stroma (perimysium) separating bundles of myofibers accompanied with acute myodegeneration due to neutrophil infiltration. Some animals surviving to scheduled necropsy had distinctive histological changes comprising myofibers loss and conspicuous myofibers regeneration lining the perimysial framework. The histological changes in the skin were observed in 1/3 females treated with GPI 15715. This animal had squamous cell hyperplasia, necrosis of the epidermal layer, neutrophilic infiltration with deep mural arteritis, and bacterial contamination. The biological relevance of this isolated finding is not known. Most of the treated animals also had tracheal lesions including sub mucosal inflammation, epithelial loss associated with metaplasia. These changes were considered compatible with mild local trauma associated with intubation.

The severity an incidence of the histological lesions were higher in myocardial tissue and skin in the GPI 15715 treated animals compared to those of the propofol treated animals. In summary, the continuous infusion with GPI 15715 (TDI = 1388 mg/kg, $AUC_{0\text{-infinity}}$ = 280 μg.h/mL, HED = 448 mg/kg) and propofol (TDI = 730 mg/kg, $AUC_{0\text{-infinity}}$ = 280 μg.h/mL, HED = 235 mg/kg) were not tolerated by the animals as indicated by mortality, acidosis, and histological lesions in the myofibers.

In the one month repeat dose toxicity study in the cynomolgus monkeys, the animals (3/sex/group) were administered with the induction dose (38 mg/kg) of the test article or formaldehyde (15.2 mg/kg) by intravenous bolus injection for 30-90 seconds followed by the intravenous infusion of GPI 15715 (42 mg/kg/hr) or formaldehyde (16.8 mg/kg/hr /day) approximately three hour per day/three times/week for one month. The toxicokinetic analysis of the GPI 15715 demonstrated its similar exposure at Day 0 and Day 28. Similarly the exposure of propofol derived from GPI 15715 at Day 0 was comparable to that of Day 28. The results indicate that there was no accumulation of the prodrug or propofol. There were no apparent gender differences in the exposure of either propofol or prodrug. The Cmax of the formate level in the body after GPI 15715 administration was comparable to that of the background formate level; however, the Cmax of the formate level in the body after formaldehyde administration was approximately 2-fold higher than that of the background formate level. The total exposures to the formate as described by AUC appeared to be slightly higher in the formaldehyde and the GPI 15715 treated group compared to that of the control. There was a decrease in the heart rate and blood pressure in the GPI 15715 treated animals. The histological findings were observed in lung, skin, kidney, heart, skeletal muscle, and trachea. There was an increased incidence of lymphoid cell aggregates in different tissues such as in the myocardium of heart, lung, femur, lacrimal gland, eyes, liver and skeletal muscle. In lungs, in addition to lymphoid cell aggregates, inflammation in the visceral pleura as well as inflammation of the lung vessels was noted. It might be possible that the lymphoid aggregates induced immune response causing inflammation in lungs. The lungs is one of the major target organs of toxicity for the test article as noted in the other toxicity studies, therefore, the manifestation of the toxicity might have exacerbated in lungs. The lymphoid aggregates in the tissues mentioned above might be an indication of inflammatory response produced by the test article. Parasitic cyst was observed at different location in the GI tract which might be an indication of immune suppression associated with the test article administration. There was a thickening of the skin surrounding the catheter insertion site in the animals administered GPI 15715; no such lesions were noted in the formaldehyde treated animals. Interestingly, a variety of histological lesions such as hemorrhage, chronic inflammation, hyperkeratosis, and squamous cell hyperplasia was noted at an increased incidence in the animals treated with GPI 15715 compared to the control animals. Thus according to the reviewer GPI 15715 at 165 mg/kg/day (HED= 53 mg/kg/day), and an $AUC_{0\text{-last}}$ =24μg.h/mL was tolerated well. However, infiltration of the lymphocyte aggregates in different tissues and skin lesions were noted after repeat dose administrations of GPI 15715 for one month.

In summary, repeat dose toxicity studies in rat, dog, and monkey did provide frank toxicity associated with GPI 15715 administrations. In rats, GPI 15715 was administered by continuous infusion of 47.5 mg/kg/hr for 1, 2, and 4 hrs for 14 consecutive days. Propofol was administered also by continuous infusion of 20 mg/kg/hr for 4 hrs for 14 consecutive days to compare its toxicity finding with the GPI 15715. The study design did not mimic the clinical scenario. Mortality was observed at high dose in GPI 15715 and in propofol treated animals. The histopathological lesions such as inflammation in lungs, cardiomyopathy, and acute inflammation in liver, bone marrow cell hyperplasia;

extramedullary hematopoesis in spleen was noted in propofol and GPI 15715 treated animals. The histological lesion were also noted in the catheter insertion sites in animals treated with GPI 15715 in much higher incidence than that of the propofol treated animals. In dogs and rats continuous administration of the test article for ≥24 hr was not tolerated as indicated by mortality (100% in dog, 33% in monkeys). The cynomolgus monkey which survived up to the scheduled necropsy showed cardiomyopathy associated with karyomegaly, inflammation in lungs, and injection site reaction. The 14-day repeat dose study in dog with a combination of bolus and infusion dose with GPI 15715 (TDI= 133 mg/kg, HED=74 mg/kg) and propofol (TDI= 51 mg/kg, HED= 28 mg/kg) resulted in a similar exposure of plasma propofol level after the administration of either GPI 15715 or propofol ($AUC_{0\text{-infinity}}$ = 24 μg.h/mL). The histological lesions in dog consisted of increased chronic active inflammation of lungs in the visceral pleural area, thickening of the injection sites in GPI 15715 treated animals than the propofol treated animals. The one month repeat dose study in the cynomolgus monkeys with GPI 15715 3x/week (165 mg/kg/day, HED= 53 mg/kg/day), and an $AUC_{0\text{-last}}$ =24μg.h/mL was tolerated; however, histopathological lesions associated with the infiltration of the lymphocyte aggregates in different tissues and skin lesions were noted. In this study, formaldehyde (TDI= 65 mg/kg/day, HED= 21 mg/kg/day) was also administered (bolus and infusion, 3x/week for one month) to compare the plasma formate level, no appreciable differences in the formate levels were noted between the GPI 15715 and formaldehyde treated animals. The formate exposure as described by $AUC_{0\text{-infinity}}$ after GPI 15715 and formaldehyde was similar to the background formate level in these animals.

Genetic toxicology: The genetic toxicity testing of GPI 15715 was completed in the bacterial mutation assay, mouse lymphoma forward mutation assay, and in vivo micronucleus assay. GPI 15715 was tested negative in the mutagenicity and in vivo clastogenicity assay in mouse. However, in the L5178YTK$^{+/-}$ mouse lymphoma cells, in the presence of metabolic activation there was an increase in the mutant frequency. The test article was therefore evaluated as positive in the presence of the metabolic activation system. This finding is concurrent with the fact that formaldehyde, the active metabolite of GPI 15715 is a known clastogen. To confirm that the in vitro clastogenicity finding with GPI 15715 was due to formaldehyde production in the presence of metabolic activation, GPI 15715 was tested in the L5178Y TK$^{+/-}$ forward mutation assay in the presence of metabolic activation with or without formaldehyde dehydrogenase (FDH). The test article was concluded to be positive for inducing forward mutation at the TK locus in L5178Y mouse lymphoma cells in the absence of FDH. The test article, however, was scored negative with metabolic activation in the presence of FDH which strengthen the hypothesis that the positive finding was due to the production of formaldehyde in the presence of the metabolic activation. The fact that clastogenicity was not observed in the in vivo assay demonstrated that the possibility of clastogenicity is minimal in similar environment where FDH might catalyze the formate produce by the metabolism of GPI 15715.

Carcinogenicity: There were no carcinogenicity studies submitted with this application. Carcinogenicity assessment is not required for the acute induction of anesthesia which is the proposed therapeutic indication for this NME.

Reproductive toxicology: GPI 15715 was studied for male and female fertility in Sprague Dawley rats, embryofetal toxicity rabbits and rats and pre and postnatal developmental toxicity in rats.

The potential effects of GPI 15715 on male fertility was studied by intravenous administration of GPI 15715 in 25 males/treatment (0, 5, 10, and 20 mg/kg) group for 4 weeks prior to mating followed by 3 weeks of the cohabitation period. The potential effects on female fertility was studied by intravenous administration of GPI 15715 in 25 females/treatment (0, 5, 10, and 20 mg/kg) group for 2 weeks prior to mating followed by 3 weeks of the cohabitation period and 1 week of gestation period. The dams were sacrificed on gestation day 13. The male and female fertility study designs are according to the OECD protocols and appeared to be valid. Female and male NOAEL=10 mg/kg/day based on clinical signs (ataxia, and decreased motor activity at 20 mg/kg, and > 10 % decrease in body weight gain at 20 mg/kg). In this study the frank toxicity for the male and female was established as indicated by a decrease in the body weight gain and clinical signs. There was decrease in the sperm count (15%) and sperm density (18%) at high dose, based on this finding, this reviewer's opinion is that the NOAEL for the male fertility is established to be 10 mg/kg (AUC_{inf} for propofol derived from GPI 15715 and GPI 15715 was 357 and 7407 ng.h/mL respectively). All other male fertility parameters such as number of day of cohabitation, rats that mated, fertility index, and rats pregnant/rats in cohabitation were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day. Therefore, the Sponsor's NOAEL in the male fertility was >20 mg/kg (AUC_{inf} for propofol and GPI 15715 were747 and 14859 ng.h/mL respectively). There were increase in the nonviable embryos (2-3 folds) at all doses, the finding was observed in all treatment groups. Based on this finding; the NOAEL for the female fertility is established to be < 5 mg/kg. The Sponsor believed that the increase in nonviable embryos are not dose related, therefore, the Sponsor's NOAEL for the female fertility was >20 mg/kg. All other female fertility parameters such as number of days of cohabitation, copulatory index, fecundity index, and fertility index were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day.

The embryo fetal development was studied in rats by administering (IV) 0, 5, 20, and 45 mg/kg/day of the test article in 25 timed pregnant dams/group from gestation days 7-17, the dams were sacrificed at Day 21. The maternal toxicity was indicated by mortality in the high dose group (2/25), and clinical signs of ataxia and decreased motor activities in all animals from the mid and high dose group. Based on these findings NOAEL for maternal toxicity was established as 5 mg/kg. This is in concurrence with Sponsor's NOAEL. There was an increase in the number of dams with resorptions. The percent of dams with any resorptions were 33, 52, 48, and 44 with 0, 5, 10, and 20 mg/kg/day dose group. There was an increase in the number of fetuses with asymmetric sternal centra and wavy ribs in the treated animals. These variations are believed to be related to the incomplete ossification. There were no such changes in the concurrent controls. In addition, there were additional central ribs in the 7^{th} vertebra in 1 fetus from the control group and 3 fetuses from the low and high dose group and 2 fetus from the mid dose group. Because of the increase in this incidence compare to that of the control, this

finding is considered as test article related. The NOAEL for fetal variations in this study could not be established and was believed to be < 5 mg/kg (AUC $_{0\text{-}17}$ for GPI 15715 and propofol were 29 and 8 μg.h/mL respectively). The Sponsor's NOAEL for fetal variation is >45 mg/kg/day.

The potential effects of GPI 15715 on embryo fetal developments was studied in rabbits by administering (IV) 0, 14, 28, 56, and 70 mg/kg/day of the test article in 20 timed pregnant dams/group from GDs 6-18, the dams were sacrificed at Day 29. The maternal toxicity was indicated by mortality dose related increase in the mortality (0, 1, 1, 2, and 6 does died at 0, 14, 28, 56, and 70 mg/kg/day respectively) and clinical signs of ataxia, decreased motor activities, impaired righting reflex, and nystagmus in the does from the test article treated group. Based on these finding, the NOAEL for maternal toxicity was established as <14 mg/kg. This is in concurrence with Sponsor's NOAEL. There was an increase in the number of does with malformations from the test article treated group. There were 3 fetuses, one in the 28 and two in the 56 mg/kg/day dose group with gross external malformations associated with the soft tissue and skeletal tissue alterations. Fetus 6550-7 in the 28 mg/kg/day dose group had domed head, cleft palate, and small tongue. Soft tissue examination revealed marked dilation of the third and lateral ventricles of the brain. Skeletal observation in this fetus showed large anterior fonatanelle, an intrafrontal in the right frontal and an incompletely ossified palate. Fetus 6564-4 in the 28 mg/kg/day dose group had two meningoceles (one in the upper lumbar region and the other in the mid lumbar region). Skeletal evaluation in this fetus showed a bifid centrum in the 5th lumbar vertebra and a displaced midline suture in the right nasals. Another fetus from the same litter, fetus 6564-6 had meningocele in the head. Skeletal evaluation revealed incompletely ossified parietal and frontal bones in the skull. There were several skeletal tissue variations in the fetuses from the test article treated does. Wavy ribs were present in one fetus (5200-9) in the 70 mg/kg/day dose group. The arches of the cervical vertebra were fused in one 28 mg/kg/day fetus (6560-7). Fused sternal centra were observed in one fetus (6525-2) from the 14 mg/kg/day dose group. One fetus in the 28 mg/kg/day dose group had an irregularly shaped ala within the scapulae. An angulated hyoid occurred in 1, 3, 11, 3, and 5 fetuses from the 0, 14, 28, 56, and 70 mg/kg/day dose groups. The increases in the 28 and 70 mg/kg/day dose group were significantly different ($p \leq 0.01$) from the vehicle control group. The increase was considered not test article related by the Sponsor because they were not dose dependent. There was a test article related increase in the irregular ossification in the skull including nasal area and the parietal and frontal bones. The percent increase in the total irregular ossification within the skull in the 0 and 70 mg/kg/day dose group were 45 and 68 respectively. One of the major changes in the nasal area in skull was the displaced midline suture. The percent increase in the displaced midline suture in the 0, 14, 28, 56, and 70 mg/kg/day dose group were 10, 16, 16, 18, and 25 respectively. The malformations of the thoracic vertebrae were observed in two fetuses. One fetus in the 14 mg/kg/day dose group had a right hemi vertebra as a 9th arch; this fetus also had centrum with attached rib. Another fetus in the high dose group, 70 mg/kg/day had a small arch in the left 11th, and fused right12 and 13th right thoracic ribs and short left 11th ribs. The NOEL for the fetal findings was established to be <14 mg/kg/day (AUC $_{0\text{-}18}$ for GPI 15751 and propofol were 76 and 11.8 μg.h/mL respectively) based on the above

mentioned findings. This is in contrast to the Sponsor's NOEL of >70 mg/kg/day based on the non dose related findings of the malformations and variations. According to the reviewer, some of the variations such as displaced midline suture, angulated hyoids, and wavy ribs are dose related and therefore directly related to the test articles. The gross external alterations are associated with malformations in the skull, vertebrae, and soft tissues and were not observed in the concurrent controls, therefore considered as test article related.

The pre and post natal development was studied in rats by administering (IV) 0, 5, 10, and 20 mg/kg/day of the test article once daily from GD 7-LD 20 or GD 24. F_1 males or females were not dosed but were likely exposed in utero. It is unknown if drug is secreted into milk. F_1 were reared to reproductive maturity. In the F_0 necropsy, one dam in the 10 mg/kg dose group had the entire litter die at LD 2. There was a slight increase in the number of pups that died between the LDs 1-14. The clinical observation from birth to postpartum Day 21 of the F_1 generation pups were limited to scabs in ear, chest mass, chest scab, nose scab in the high dose group. The biological significance of such findings is unknown. The pup mortality between LDs 1- 21 was higher in the high dose group animals. In the C-section delivery from F_1 dams observations such as corpora lutea, implantations, litter sizes, and percent male fetuses were comparable among the four maternal dose groups and did not differ significantly. However, the number of dams with any resorptions increased dose dependently. The percent of dams with any resorptions were 20, 36, and 54 in the 5, 10, and 20 mg/kg/day. Based on the resorptions findings in the F_2 females, the NOAEL was determined to be 10 mg/kg/day (HED = 1.6 mg/kg).

The reproductive toxicity studies were conducted in rats (male and female fertility, embryofetal toxicity and pre and post natal toxicity) and rabbits (embryofetal toxicity). The major findings were the resorptions of the fetus in rats and rabbits in all of the studies conducted. In addition, incomplete ossifications were noted in rat and rabbit embryofetal development studies. In rats, there was an increase in the number of fetus with asymmetric sternal centra and wavy ribs in the treated animals. There were no such changes in the concurrent controls. In addition, there was an additional central ribs in the 7^{th} vertebra in 1 fetus from the control group and 3 fetuses from the low and high dose group and 2 fetus from the mid dose group. Because of the increase in this incidence compare to that of the control, this finding in considered as test article related. In rabbits, dose related skeletal variations such as displaced midline suture, angulated hyoids, and wavy ribs were noted and therefore considered related to the test articles. In rabbits malformations were also noted. There were 3 fetuses, one in the 28 and two in the 56 mg/kg/day dose group with gross external malformations associated with the soft tissue and skeletal tissue alterations. Fetus 6550-7 in the 28 mg/kg/day dose group had domed head, cleft palate, and small tongue. Soft tissue examination revealed marked dilation of the third and lateral ventricles of the brain. Skeletal observation in this fetus showed large anterior fonatanelle, an intrafrontal in the right frontal and an incompletely ossified palate. Fetus 6564-4 in the 28 mg/kg/day dose group had two meningoceles (one in the upper lumber region and the other in the mid lumber region). Skeletal evaluation in this fetus showed a bifid centrum in the 5^{th} lumbar vertebra and a displaced midline

suture in the right nasals. Another fetus from the same litter, fetus 6564-6 had meningocele in the head. Skeletal evaluation revealed incompletely ossified parietal and frontal bones in the skull.

Local tolerance: The effect of GPI 15715 on hemolysis, irritation in eye, skin, vascular, and perivascular was evaluated by several *in vitro* and *in vivo* analyses. The hemolytic potential of the compound was evaluated in four different *in vitro* assays. The hemolytic potential of the test article increased with the increasing concentration. The hemolytic potential of GPI 15751 (1 mg/mL) and propofol (0.05 mg/mL) was 3.1 and 25.8 % respectively under the validated testing condition (American Society for Testing Material-direct contact method). The result indicates that fospropofol is expected to be minimally hemolytic; however, the hemolytic potential of propofol, the marketted product, is much higher than that of GPI 15715.

The effect of GPI 15715 on the ocular irritation was assessed in rabbits (GLP) by administering 0.1 mL of the test article (35 mg/mL) directly on one eye of the animal (n=6). The irritation in the treated and the control eye were measured in Draize scale up to 3-days. No ocular irritation was noted indicating that the test article is not an ocular irritant under this experimental condition.

The effect of GPI 15715 on skin irritation was also assessed in rabbits (GLP) by applying the 0.5 mL of the test article (35 mg/mL) directly on the abraded skin of the animal (n=6). The irritation in the intact and the abraded skin were examined up to 3-days. No skin irritation was noted indicating that the test article is not a skin irritant under this experimental condition.

The effect of GPI 15715 on vascular irritation was assessed in rabbits (GLP) by a single intravenous administration of 0.1 mL of the test article (35 mg/mL) directly in the vein in right ear of the animal (n=10). The irritation in the veins of the treated and the untreated ear were measured up to 8 days. No test article related irritation was noted indicating that GPI 15715 is not a vascular irritant under this experimental condition.

The effect of GPI 15715 on perivascular irritation was assessed in rabbits (non GLP) by a single intravenous administration of 0.1 mL of the test article (35 mg/mL) around the perivascular area in the right ear of the animal (n=6). The irritation surrounding the veins of the treated and the untreated ear were measured up to 11-days. No test article related irritation was noted indicating that GPI 15715 is not a perivascular irritant under this experimental condition. In this study, propofol was also tested; it was found to be moderately irritating in the perivascular area as observed by the appearance of edema and erythema.

The effect of GPI 15715 on local irritation was also studied in rats (n=3) by administering it subcutaneously. The doses used were 10, 35, 100, 200 mg/kg at a concentration of 1 mL/kg, 45 and 150 mg/kg at a concentration of 0.3 mL/kg, and 60 mg/kg at a concentration of 200 mg/mL. The test article produced local irritation characterized by acute inflammation at 60 mg/kg and 200 mg/kg indicating concentration dependent

irritation potential of the test article under this experimental condition. A NOAEL of 100 mg/kg (HED = 10.6 mg/kg) at a concentration of 1 mg/mL was established in this study.

Special toxicology: There were two special toxicity studies that were conducted with GPI 15715. The hypersensitivity of the test article was tested in a dermal sensitization study in guinea pig (n=5) using standard Buehler design. There was no indication of contact sensitization in the guinea pig after the administration of GPI 15715 in the induction and the challenge phase. The oral toxicity of GPI 15715 was also assessed in a 7-day repeat dose toxicity study in Sprague Dawley rats (5/sex/group). The doses used were 0, 4, 20, 50, and 100 mg/kg. The clinical observations of flat postures were noted in females at Days 2 and 4 but not in males. Based on these observations a NOAEL of 100 mg/kg was established for oral toxicity in rats.

2.6.6.2 Single-dose toxicity

Study title: Acute Toxicity of GPI 15715 in Sprague-Dawley Rats and CD-1 Mice

Key study findings

- This is a dose range finding study for obtaining an optimum bolus dose for intravenous administration of GPI 15715 in rats and mice. No mortality was observed in the rats and mice up to 80 and 160 mg/kg respectively.
- Clinical sign of sedation indicated by loss of righting reflex was noted in rats at ≥ 80 mg/kg but not in mice indicating mice are less sensitive than rats to GPI 15715 induced anesthesia.

Study number: Single dose tox\3000-15715-00-04g
Volume # and page #: Module 4-eCTD submission; Page#: 1-101
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 07-31-2000
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15751, 21708-21-3, 84.8%
Vehicle: 0.9% saline

Study design: Rats and mice 5/sex/group were administered with the test article via a single bolus IV injection in the tail vein as indicated in the Sponsor's study design table. The parameters evaluated were mortality, body weights, food consumption, and gross pathology.

Study design table

Group	Treatment	Test Article Dosage mg/kg	Animal Numbers	
			Rats	Mice
1	vehicle control	0	11126-11135	11176-11185
2	GPI-15715	40	11136-11145	11186-11195
3	GPI-15715	80	11146-11155	11196-111205
4	GPI-15715	160	11156-11165	11206-11215
5	GPI-15715	320	11166-11175	11216-11225

Results: The high dose 320 mg/kg was fatal to both rats and mice. The next high dose 160 mg/kg was fatal to 3/5 rats (both males and females) but not to the mice. No mortality was observed at ≤ 80 mg/kg. A loss of righting reflex was noted in rats but not in mice at 80 mg/kg indicating that mice are less sensitive than rats to GPI 15715.

Study title: Propofol and GPI 15715: A Pilot 8-Hour Anesthesia Study in the Rat

Key study findings

- This is a pilot study to evaluate the different bolus and infusion doses of GPI 15715 for the induction and maintenance of anesthesia. Propofol was used as comparator in this study.
- All animals (Sprague Dawley rats, n=2) died following infusion of GPI 15715 (70-140 mg/kg/hr) and propofol (30-40 mg/kg/hr) for 8 hrs.
- The intravenous bolus administration either with propofol (5, 10, 15 mg/kg) or with GPI 15715 (20, 40, and 60 mg/kg) did not cause any mortality.
- Clinical pathological changes such as decreased WBC counts, potassium, calcium, phosphorous, and an increased urea nitrogen and creatinine were noted in the GPI 15715 treated animals (n=1).

Study number: Single dose tox\3000-15715-01-02n
Volume # and page #: Module 4-eCTD submission; Page#: 1-134
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 05-16-2000
GLP compliance: No
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, 11228-41, 86.29%
Vehicle: 0.9% saline

Study design: The anesthetic potential of GPI 15715 was compared to that of propofol in this pilot study. To evaluate the induction of anesthesia, propofol (5, 10, and 20 mg/kg/day) and GPI 15715 (20, 40, and 60 mg/kg/day) were administered via single bolus intravenous administrations (1 animal/sex/group). To evaluate the maintenance of anesthesia a median dose of propofol (35.96 mg/kg/hr) and GPI 15715 (115.7 mg/kg/hr) were administered via continuous intravenous infusion for 8 hrs (2 animals/sex/group) as

indicated in the Sponsor's study design table. The parameters observed were clinical signs, hematology, clinical pathology, gross lesions, and microscopic evaluation.

Study design table

Group	Test Article	Route of Administration	Daily Dose: Dose (mg/kg)[b] or (mg/kg/hour)[c]	Daily Dose: Volume (mL/kg)[b] or (mL/kg/hour)[c]	Daily Dose: Conc. (mg/mL)	No. of animals: Initial M	Initial F	Terminal Blood Collection: Clinical Pathology M	Clinical Pathology F	TK, Blood Gas, Ionized Calcium M	TK, Blood Gas, Ionized Calcium F	Necropsy[a] M	Necropsy[a] F	Microscopic Pathology M	Microscopic Pathology F
1	Propofol	IV bolus	5	0.5	10	1	1	0	0	0	0	0	0	0	0
2	Propofol	IV bolus	10	1.0	10	1	1	0	0	0	0	0	0	0	0
3	Propofol	IV bolus	15	1.5	10	1	1	0	0	0	0	0	0	0	0
4	GPI 15715	IV bolus	20	1.0	20	1	1	0	0	0	0	0	0	0	0
5	GPI 15715	IV bolus	40	2.0	20	1	1	0	0	0	0	0	0	0	0
6	GPI 15715	IV bolus	60	3.0	20	1	1	0	0	0	0	0	0	0	0
7	Propofol	IV infusion	30-40[d]	3-4[d]	10	2	2	1	1	1	1	2	2	2	2
8	GPI 15715	IV infusion	70-140[d]	3.5-7[d]	20	2	2	1	1	1	1	2	2	2	2

[a]Animals in Groups 7 and 8 were sacrificed after up to 8 hours of infusion; recovery was not observed. Animals were sacrificed by exsanguination during collection of terminal blood samples. Animals in Groups 1-6 were sacrificed after recovery; necropsy was not performed.

[b]Dose units for IV bolus for Groups 1 through 6.

[c]Dose units for IV infusion for Groups 7 and 8.

[d]Animals received an induction dose of Propofol or GPI 15715, followed by the maintenance dose administered via infusion. Animals were dosed to effect, i.e., light sedation.

M = Male; F = Female; mg/kg = milligrams of test article per kilogram of body weight

The first day of dosing was defined as Day 0 of the study.

Results: All animals (except one male in the propofol group) died during the infusion; therefore the assessment of the maintenance of anesthesia could not be evaluated. The animals administered with the bolus dose of GPI 15715 showed deeper anesthesias compared to that of the propofol as indicated by the sensory reflex. Clinical pathological changes associated with decreased WBC counts, potassium, calcium, phosphorous, and an increased urea nitrogen and creatinine were noted in the test article treated animals.

Study [redacted]458007: A Pilot 6-Hour Infusion Toxicity Study of GPI 15715 (Aquavan®) in Beagle Dogs b(4)

Key study findings

- This is a pilot infusion dose range finding study. The dogs (n=3 males/group) were induced for anesthesia with an intravenous bolus administration of 38 mg/kg, the animals were then maintained in an anesthetic state for 6 hrs with an intravenous infusion dose of 70, 80 or 90 mg/kg/hr.
- Clinical signs of anesthesia as indicated by mucus membrane color and capillary refill time, pupil size and response to light and reflex by touching hairs close to eyes, oral pharyngeal reflex: checking for jaw tension, ear pinna reflex, and pedal reflex was noted in all animals.
- The increase in AUC of GPI 15715 and propofol following 70, 80, and 90 mg/kg/hr of GPI 15715 administrations were 358, 370, and 371 µg.h/mL and 41, 63, 64 µg.h/mL, respectively suggesting a non-dose-related effect between the

mid and high dose. The Cmax of GPI 15715 and propofol following 70, 80, and 90 mg/kg/hr of GPI 15715 administrations were 89.3, 144.3, and 171 µg/mL and 8, 11.9, 12.5 respectively. The formate levels after both of the test articles administrations were similar to that of pre dose levels.

- There was no mortality; however, one animal from the high dose group was dropped from the study due to a drop in blood pressure. The decrease in heart rate and blood pressure were noted in all treated animals. A prolongation in QTc interval was noted > 20 secs in all low dose animals up to 26 hrs post loading dose, mid and high dose animals up to 6 hrs post loading dose. QTc times were recovered by 50 hrs post dosing. Inappropriate ventilation might have been responsible for this increase in the QTc time.
- No NOAEL could be established due to the QTc finding in the low dose (TDI=458 mg/kg, HED= 254 mg/kg) animal under this experimental condition.

Study number: Single dose tox\wil-458007
Volume # and page #: Module 4-eCTD submission; Page#: 1-618
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 11-07-03
GLP compliance: No
QA reports: Yes
Drug, lot #, and % purity: GPI 15715 (38 mg/mL), 176I0603, potency 101%
Vehicle: The test article was formulated by the Sponsor and used as such by the CRO; no vehicle control was used in this experiment.

Study design: To evaluate the safety and tolerability of GPI 15715, beagle dogs 3 males/groups were induced for anesthesia using 38 mg/kg as a bolus intravenous (cephalic vein) dose; the dogs were subsequently sedated for an additional 6 hrs by intravenous infusion with the rate of 70, 80, and 90 mg/kg/hr over a 6 hr time period and necropsied at 2-3 days post loading dose. The Sponsor's study design is reproduced in the following table. The animals were observed for two days following the test article infusion and then necropsied.

Study design table

Group Number	Test Article	Nominal Dosage Level (mg/kg/hr)	Range of Total Dose Received (mL)	Dosage Volume (mL/kg/hr)	Dosage Concentration (mg/mL)	Number of Animals
1	GPI 15715	70.0	102.0-111.29	2.0	35.0	3
2	GPI 15715	80.0	111.44-140.31	2.3	35.0	3
3	GPI 15715	90.0	131.23-144.9	2.6	35.0	2

Results: All dogs survived to the scheduled necropsy. Clinical observations including abnormal excreta were noted in all animals at all dose groups. In addition, emesis was noted in all animals in the high dose group. The clinical signs of anesthesia as indicated

by mucus membrane color and capillary refill time, pupil size and response to light and reflex by touching hairs close to eyes, oral pharyngeal reflex: checking for jaw tension, ear pinna reflex, and pedal reflex was noted in all animals. A reduction in the body weights were noted in all test article treated animals. The average body weight loss at low, mid, and high dose groups were 0.33, 2.49, and 5.18% respectively. The body weight loss was associated with a reduction in food consumption of 17, 64, and 85% at low mid and the high dose group respectively. There was a decrease in blood pressure, heart rate, and respiratory rate. One animal from the high dose group was removed from the study due to a large drop in the blood pressure at 280 mins. The mean heart rate changes are decreased in the test article treated animals (refer to Sponsor's table).

Effect of GPI 15715 on heart rate

Selected Mean Heart Rate Values - % Difference From Baseline

Group	Baseline	15-Minutes Post-Loading Dose		26 Hours Post-Loading Dose		50 Hour Post-Loading Dose	
	bpm	bpm	% change from baseline	bpm	% change from baseline	bpm	% change from baseline
70 mg/kg/hr	106	114	7.5%	105	-0.9%	103	-2.8%
80 mg/kg/hr	187	179	-4.3%	146	-21.9%	116	-38.0%
90 mg/kg/hr	105	158	50.5%	75	-28.6%	114	8.6%

There was a prolongation of the QT/QTc (refer to Sponsor's QTc table) in animals from all dose groups during the infusion period. The heart rat corrected QTc values are shown in the table below. Highest increase in the prolongation time was noted with the lowest dose and thus the effect is not related to the dose administered. It also showed sign of recovery by 50 hrs post loading dose.

Effect of GPI 15715 on QTc

	QTcV (milliseconds)		
	70 mg/kg/hr	80 mg/kg/hr	90 mg/kg/hr
Pretest	223.34	218.89	225.69
Predose	223.11	223.77	221.17
2 hours post-loading dose	273.44	258.90	246.77
4 hours post-loading dose	263.91	271.35	260.53
5.5 hours post-loading dose	271.08	263.79	252.60
26 hours post-loading dose	240.84	217.22	218.14
50 hours post-loading dose	237.33	234.31	210.58

There was a slight reduction in the red blood cell counts, hemoglobin and hematocrit counts, and slight reduction in the white blood cells. There was a slight increase in the partial CO_2 pressure indicating (21-26 mmHg at 70 mg/kg/hr group, 26-31 mmHg at 80 mg/kg/hr group, 30-33 mmHg at 90 mg/kg/hr group) changes in the respiratory centers resulting in insufficient alveolar ventilation and CO_2 accumulation.

There were no microscopic or macroscopic effects related to the test article administration. Also there were no test article related ophthalmological changes.

There was an increase in exposure levels as indicated by the Cmax and AUC after GPI 15715 administrations but the exposure was not always dose related (refer to Sponsor's table. The increase in AUC of GPI 15715 and propofol following 70, 80, and 90 mg/kg/hr of GPI 15715 administrations were 358, 370, and 371 µg.h/mL and 41, 63, 64 µg.h/mL respectively suggesting a non-dose related effect between the mid and high dose

The Cmax of GPI 15715 and propofol following 70, 80, and 90 mg/kg/hr of GPI 15715 administrations were 89.3, 144.3, and 171 µg/mL and 8, 11.9 and 12.5, respectively. There were no changes in the formate level from the pre dose to post dose levels after GPI 15715 administration.

Summary of toxicokinetic analysis

Analyte	Group	Initial IV Bolus Dose of Aquavan[a] (mg/kg)	6 hr IV Infusion Rate of Aquavan[a] (mg/kg)	N	C_{max} (µg•h/mL)	AUC_t (µg•h/mL)	T_{max}[a,b] (hr)
GPI 15715	1	38	70	3	89.3 (14.1)	358 (3.1)	NC
	2	38	80	3	144.3 (31.6)	370 (7.1)	NC
	3	38	90[d]	2	171 (146-196)[c]	371 (363-380)[c]	NC
Propofol	1	38	70	3	8.24 (8.1)	41.9 (7.8)	3.02 (3.00-6.03)
	2	38	80[d]	3	11.9 (11.6)	63.5 (12.0)	6.00 (3.00-6.00)
	3	38	90[e]	2	12.5 (10.0-14.9)[c]	64.2 (53.3-75.0)[c]	3.03 (3.02-3.02)[c]

[a] = Median (range)
[b] = For GPI 15715, T_{max} was at the first measured time point and is not reported
[c] = Reported (range) as N<3
[d] = One of the dogs in the 80 mg/kg/hr group received infusion for < 6 hrs (total infusion time 5 hours 12 minutes)
[e] = The two dogs in the 90 mg/kg/hr group received infusion < 6 hrs (total infusion time 4 hours 40 minutes and 5 hours 35 minutes)

Study title: GPI 15715 and Propofol: A Pilot Intravenous Infusion Study in Cynomolgus Monkeys

Key study findings

- This is a pilot study to determine the optimum bolus dose for GPI 15715 for the induction of anesthesia and maintenance of anesthesia in cynomolgus monkeys (1/sex/group). The general condition of anesthesia after GPI 15715 and propofol were compared in a cross over study with a 7-day washout period.
- GPI 15715 at an intravenous induction dose of 45.5 mg/kg and infusion of up to 64 mg/kg/hr for 6 hrs were well tolerated in monkeys. Clinical signs of anesthesia were noted in all animals. The only adverse events observed were decrease in the blood pressure and heart rate.

- The total dose GPI 15715 administered/day was 472.2 mg/kg (HED=152 mg/kg) for the male (body weight =3.1 kg) and 410 mg/kg (HED=132 mg/kg) for the female (body weight 2.6 kg) which is theoretically equivalent to 248.5 (HED=80 mg/kg) and 216 mg/kg (HED=70 mg/kg) for the male and female respectively.

Study number: Single dose tox\3000-15715-01-01n
Volume # and page #: Module 4-eCTD submission; Page#: 1-111
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 02-02-2001
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715, 12164-G1-00-001, 93.3%
Vehicle: 0.9% saline
Positive control: Propofol (10 mg/mL emulsion containing 100 mg/mL soybean oil, 22.5 mg/mL glycerol, and 12 mg/mL egg lecithin), lot #00L306.

Study design: This is a dose range finding study to obtain the optimal dose for the induction and maintenance of anesthesia for a 6-hr period in the cynomolgus monkeys (1/sex/dose). The monkeys were first induced with 10 mg/kg propofol, IV bolus for the initiation of anesthesia followed by a maintenance dose of 34-37.3 mg/kg/hr, IV infusion for 6-hrs. After a wash–out period of one week an effective induction dose (38 and 45.6 mg/kg/hr) for GPI 15715 was determined. On the following day an induction dose of 38 or 45.6 mg/kg/hr IV bolus was followed by 65-71 mg/kg/hr for 6-hrs via IV infusion for the maintenance of the anesthesia for 6-hrs (refer to Sponsor's study design table). The animals were monitored for clinical signs, cardiovascular assessments, blood gas analyses, and gross macroscopic lesions.

Study design table

Group	Study Day	Induction Dose			Maintenance Dose[a]			Number of Animals	
		Dose	Vol.	Conc.	Dose	Flow Rate	Conc.	Initial	
		mg/kg	mL/kg	mg/mL	mg/kg/hour	mL/kg/hr	mg/mL	M	F
1 (Propofol)	0	10	1.0	10	-	-	-	1	1
	1	10	1.0	10	34.0 – 37.4	3.4	10	1	1
2 (GPI 15715)	7	38	1.9	20	-	-	-	1	1
	8	45.6	2.28	20	64.4 – 71.1	3.23 – 3.56	20	1	1

[a]The maintenance dose was adjusted during the study to maintain the proper plane of anesthesia. Adjustments were made by changing the flow rate.

M = male; F = female; Vol. = volume; Conc. = concentration

The day of dose initiation was defined as Day 0 of the study.

Results: The study determined the efficacious dose of GPI 15715 for the induction and the maintenance of anesthesia. The total dose GPI 15715 administered was 472.2 mg/kg for the male (body weight =3.1 kg) and 410 mg/kg for the female (body weight 2.6 kg)

which is theoretically equivalent to 248.5 and 216 mg/kg for the male and female respectively. The total dose of the propofol administered was 233.7 and 233 mg/kg in the male and the female respectively. There were clinical signs of anesthesia in both of the animals after propofol and GPI 15751 administration. A decrease in the heart rate and blood pressure were noted in all animals. No other adverse events were observed.

TABLE 1. Toxicokinetic Parameters of Propofol Following Intravenous (Bolus) Administration of Propofol Injectable Emulsion to Monkeys (Day 0)

Animal	Sex	T_{max} (min)	C_{max} (µg/mL)	AUC_{0-t} (min*µg/mL)	$AUC_{0-\infty}$ (min*µg/mL)	CL (ml/min/kg)	$T_{1/2elim}$ (min)
7740	M	1.0	7.894	85.27	89.15	112.2	6.8
7843	F	1.0	2.875	50.92	70.66	141.5	15.9

TABLE 2. Toxicokinetic Parameters of Propofol Following a Bolus and a 6-Hour Intravenous Infusion of Propofol Injectable Emulsion to Monkeys (Day 1)

Animal	Sex	T_{max} (min)	C_{max} (µg/mL)	AUC_{0-t} (min*µg/mL)	$AUC_{0-\infty}$ (min*µg/mL)	CL (ml/min/kg)	$T_{1/2elim}$ (min)
7740	M	240.0	18.87	3964	4008	53.39	65.1
7843	F	360.0	21.04	4985	5005	42.76	43.9

TABLE 3. Toxicokinetic Parameters of GPI 15715 and Propofol Following Intravenous Administration (Bolus) of GPI 15715 to Monkeys (Day 7)

Animal	Sex	T_{max} (min)	C_{max} (µg/mL)	AUC_{0-t} (min*µg/mL)	$AUC_{0-\infty}$ (min*µg/mL)	CL (ml/min/kg)	$T_{1/2elim}$ (min)
				GPI 15715			
7740	M	1.0	574.8	1874	1878	20.24	8.7
7843	F	1.0	551.5	1451	1452	26.18	6.3
				Propofol			
7740	M	5.0	6.806	195.6	256.9	NA[1]	29.4
7843	F	1.0	4.728	153.3	176.2	NA	19.4

[1]NA – Not Applicable

Study title: GPI 15715 and Formaldehyde: An Acute Intravenous Toxicity Study and Toxicokinetic Study in Cynomolgus Monkeys

Key study findings

- This is a dose range-finding study to obtain the maximum tolerated dose for GPI 15715, the doses used were 38, 44, 50, and 56 mg/kg, same animals (n=2/sex/group) were used in the study for different doses. The animals were sacrificed 7-days after the final dose administration. Formaldehyde 20 mg/kg were also administered in the monkeys (n=2/sex/group) to compare the formate production followed by GPI 15715 and formaldehyde.

- GPI 15715 induced anesthesia within 2-4 mins in all animals at all doses as evaluated by the assessment of reflex.
- All animals showed a decrease in the heart rate and MAP after GPI 15715 administration. There were no changes ECG and QT prolongation after the intravenous administration of GPI 15715.
- The toxicokinetic analyses of the formate produced after the GPI 15715 and formaldehyde administration showed that the Cmax of formate after formaldehyde administration was higher. However, the overall systemic exposure as indicated by the AUC was similar in the animals administered either with formaldehyde or GPI 15715.

Study number: Single dose tox\3000-15715-02-01g
Volume # and page #: Module 4-eCTD submission; Page#: 1-580
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 02-07-2002
GLP compliance: Yes
QA reports: Yes
Drug and lot #: GPI 15751 (19.2 mg/mL), CBL 1214-10
Vehicle: The test article was supplied by the Sponsor in saline solution and was used by the CRO as supplied. No vehicle control was used in this study.

Study design: The study was designed to find out the maximum tolerated dose for GPI 15715 after a single intravenous bolus administration and usually same animals (1-2/sex/group) were used for different doses. The doses used were 38, 44, 50, and 56 mg/kg. The animals were monitored for clinical signs, cardiovascular assessments, clinical pathology, and opthalmology. For toxicokinetic analysis, 2 animals/sex/group was used for assessing the pharmacokinetics of GPI 15715 (50 mg/kg) and formaldehyde (20 mg/kg). After 7 days of the final treatment, the animals were sacrificed and macroscopic and microscopic evaluations were conducted (refer to Sponsor's study design table).

Study design table

	Daily Dose[a]			Number of Animals	
Group	Dose (mg/kg)	Volume (mL/kg)	Concentration (mg/mL)	Males	Females
1	38	2.0	19.2	1	1
2	44	2.3	19.2	1	1
3	50	2.6	19.2	2[b]	1
4	56	2.9	19.2	1	1

[a]Dose represents active ingredient.
[b]An additional male was added to Group 3 to provide additional information at 50 mg/kg.
mg/kg = milligrams of test article per kilogram of body weight

Results: GPI 15715 induced anesthesia within 2-4 mins in all animals as evaluated by the assessment of reflexes. There were no appreciable changes in the hematology, clinical chemistry, opthalmology, macroscopic, and microscopic evaluation after a single intravenous bolus administration of GPI 15715 (50 mg/kg) and formaldehyde (20 mg/kg) in the monkeys. All animals showed a decrease in the heart rate and MAP after GPI 15715 administration. There were no changes in ECG or evidence of QT prolongation after the intravenous administration of GPI 15715. The toxicokinetic analyses of the formate produced after the GPI 15715 and formaldehyde administration showed that the Cmax of formate after formaldehyde administration was higher. However, the overall systemic exposure to formate as indicated by the AUC was similar in the animals administered either formaldehyde or GPI 15715.

Summary of toxicokinetics analyses

8.1 Table 1. Overall Mean (S.D.) Toxicokinetic Parameters for GPI 15715 and Formate

PHASE	GROUP (TEST ARTICLE) (DOSE) (n = 2 Males & 2 Females)	COMPONENT	C_{max} (µg/mL) (S.D.)	AUC_{0-t} (µg.h/mL) (S.D.)	$T_{1/2}$ (h) (S.D.)	CL (L/h/kg) (S.D.)	Vd (L/kg) (S.D.)	T_{max}** (h)	Time to return to baseline** (h)
1	1 (GPI 15715) (50 mg/kg)	GPI 15715	122 (44)	19.8 (7.6)	0.25 (0.01)	2.62 (1.61)	0.966 (0.607)	NE	NE
		FORMATE (Baseline Normalized)	13.7 (1.6)	49.6 (41.1)	NE	NE	NE	0.25 (0.08 – 0.25)	0.75 (0.12 – 1.00)
		FORMATE (Original)	41.3 (2.3)	649 (55)	NE	NE	NE	0.25 (0.08 – 0.25)	NE
	2 (FORMALDEHYDE) (12.3 mg/kg)	FORMATE (Baseline-normalized)	32.0 (4.8)	49.9 (14.6)	NE	NE	NE	0.18 (0.12 – 0.25)	1.50 (1.00 – 24.00)
		FORMATE (Original)	56.7 (9.1)	619 (88)	NE	NE	NE	0.18 (0.12 – 0.25)	NE
2	1* (FORMALDEHYDE) (20 mg/kg)	FORMATE (Baseline-normalized)	53.1 (4.3)	51.5 (20.0)	NE	NE	NE	0.12 (0.03 – 0.17)	4.00 (0.08 – 24.00)
		FORMATE (Original)	77.9 (5.4)	556 (188)	NE	NE	NE	0.12 (0.03 – 0.17)	NE
	2 (GPI 15715) (50 mg/kg)	GPI 15715	119 (65)	21.3 (11.7)	0.37 (0.11)	3.83 (4.72)	2.60 (3.85)	NE	NE
		FORMATE (Baseline-normalized)	6.65 (1.10)	28.9 (19.2)	NE	NE	NE	4.50 (0.25 – 8.00)	13.00 (0.50 – 24.00)
		FORMATE (Original)	33.6 (4.0)	655 (99)	NE	NE	NE	4.50 (0.25 – 8.00)	NE

* n = 5 (2 Males & 3 Females); ** as median (range); NE – Not Estimated

2.6.6.3 Repeat-dose toxicity

Study title: Fourteen-Day Toxicity Study of GPI 15715 in Sprague-Dawley Rats

Key study findings

- The Sprague Dawley rats (5/sex/group) were administered with a continuous infusion of 47.5 mg/kg/hr for 1, 2, or 4 hrs for fourteen consecutive days with GPI

15715. Propofol (20 mg/kg/hr) was administered by continuous infusion for 4 hrs and the toxicity of GPI 15715 and propofol was compared.

- The toxicokinetic analysis of GPI 15715 and propofol derived from it showed extreme variability. There was no apparent accumulation of propofol following the repeat dose administration in rat. No gender differences were noted. The elimination half life ranged between 0.3-1.5 hrs indicating rapid elimination.
- There were 2 mortalities each with propofol and high dose GPI 15715, the cause of death is unknown.
- The histopathological lesions after GPI 15715 administration was associated with chronic inflammation of lungs, acute inflammation in liver, cardiomyopathy, bone marrow cell hyperplasia in femur, extramedullary hematopoesis in spleen, and congestion in kidney at all doses.
- The incidence of lung lesions were higher than the controls, however, not dose related and was described to be associated with infiltration of foreign particles such as hair and skin structures.
- Increased incidence of cardiomyopathy in the heart was noted in GPI 15715 treated animals with 2 hrs and 4 hrs of continuous infusion/day compared to those of the controls. The severity index was minimal in all animals except 2 females with 2 hrs infusion regimen where the severity was described as moderate.
- There was an increased incidence of acute inflammation in liver characterized as minimal to mild in severity in the test article treated animals compared to those of the controls. Similar changes were noted in the propofol treated animals.
- There was an increased incidence of congestion in the kidney; no such changes were noted in the control animals, however, similar changes were noted in the propofol treated animals.
- There was an increased incidence of lesions in the injection sites in the test article treated animals compared to those of the controls and the propofol treated animals.
- In addition, an increase incidence of bone marrow cell hyperplasia in femur and extramedullary hematopoesis were also noted in the test article treated animals, these lesions were also considered test article related, similar changes were, however, noted in the propofol treated animals.
- No NOAEL could be determined due to the injection site findings at low dose, 47.5 mg/kg (HED=7.6 mg/kg, $AUC_{(0\text{-}4\ h)}$ ~15 µg.h/mL).

Study number: Study 3000-15715-00-07g
Volume # and page #: Module 4-eCTD submission; Page#: 1-269
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 08-08-2000
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, 21708-21-3, 84.8%
Vehicle: 0.9% saline
Positive control: Propofol, lot #s 57-921-27 & 58-850-Z7, purity not mentioned

Methods

Doses: GPI 15715 47.5 mg/kg/hr for 1, 2, or 4 hrs;
Propofol 20 mg/kg/hr for 4 hrs
Species/strain: Sprague Dawley [redacted]: CD®BR rats b(4)
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: The test article and the positive controls were administered via slow infusion, the volume of the injection, and the infusion rate for the main study were adjusted in a dose range finding study as described in the study design below.
Satellite groups used for toxicokinetics or recovery: There were no satellite group for toxicokinetic analyses; blood was collected from all test article treated animals at Day 14.
Age: 9-10 weeks
Weight: Males: 244.9-391.6 g; Females: 216.9-273.1 g
Sampling times: The blood samples for the toxicokinetics were collected from the animals in the dose range finding study on Day 4 and from the animals in the main study on Day 14.
Unique study design or methodology (if any): The study was conducted in two phases. In the first phase, the rate of infusion of GPI 15715 was adjusted in every 30 mins interval to determine a dose range for long term infusion of GPI 15715. The goal for adjusting the rate of infusion is to maintain the animal at a level of light sedation where the righting reflex was lost but toe pinch response remain. The dose range finding study was conducted for four days. In the second and the main phase of the study, animals were dosed daily for fourteen days by continuous infusion with either propofol or GPI 15715 for one, two, or four hours per day. The Sponsor's tables for the dose range finding study and the main study are reproduced as follows.

Dose Range-Finding Phase

Group	Test Article	Day 1 Dosage (mg/kg)	Number of Animals Male	Number of Animals Female	Animal Numbers Male	Animal Numbers Female
1	Propofol	20	2	2	11226-11227	11228-11229
2	GPI 15715	30	2	2	11230-11231	11232-11233

Main Study Phase

Group	Test Article	Dosing Interval (hour)	Number of Animals Male	Female	Animal Numbers Male	Female
1	Vehicle Control	4	5	5	11234-11238	11239-11243
2	Propofol	4	5	5	11244-11248	11249-11253
3	GPI 15715	1	5	5	11254-11258	11259-11263
4	GPI 15715	2	5	5	11264-11268	11269-11273
5	GPI 15715	4	5	5	11274-11278	11279-11283

Observations times and results

Mortality: In the dose range finding study, 2/2 males died in the propofol treated group on Day 2 and ½ males died in the GPI 15715 treated group on Day 3.
In the main study, 2/5 males and 2/5 females died after 4 hrs of propofol infusion between Days 13-14. On the contrary, 1/5 males died after continuous infusion with 47.5 mg/kg/hr GPI 15715 for one hour at Day1, the animal was replaced. In addition, 1/5 males and 1/5 females died after continuous infusion with 47.5 mg/kg/hr GPI 15715 for four hour at Day 10. The unscheduled necropsy of these animals did not show any definitive cause for death.

Clinical signs: Detailed clinical signs were observed prior to dosing on Day 1 and Days 4, 7, 10, and 13 and on the day of termination. There were no test article related changes in the clinical signs other than sedation. No sedation was observed with 47.5 mg/kg/hr GPI 15715 infused for one hour. Light level of sedation was observed 2 hrs posts dosing with 47.5 mg/kg/hr infusion. The swelling of injection sites were noted in most of the control and the test article treated animals.

Body weight: Body weights were recorded prior to dosing on Day 1 and on Days 4, 7, 10, and 13 and on the day of termination. There were no changes in the body weights.

Food consumption: The food consumption was recorded on Days 1-4, 4-7, 7-10, 10-13, and 13-14. There was a test article related decrease in the food consumption, however, there were no changes in the body weights. Therefore it may be assumed that the decrease in food consumption is compensated with less expenditure of energy due to light sedation.

Hematology and coagulation: The blood samples were collected from all animals on Day 15 prior to termination and following parameters were analyzed. There were no test article related changes in the hematology and coagulation parameters.

Hematology/Coagulation

leukocyte count (WBC)	leukocyte differential
erythrocyte count (RBC)	cellular morphology
hemoglobin (HGB)	mean corpuscular volume (MCV)
hematocrit (HCT)	mean corpuscular hemoglobin (MCH)
platelet count (PLT)	mean corpuscular hemoglobin concentration (MCHC)
mean platelet volume (MPV)	
prothrombin time (PT)	activated partial thromboplastin time (APTT)

Clinical chemistry: The blood samples were collected from all animals on Day 15 prior to termination and following parameters were analyzed. There were no test article related changes in the clinical pathology.

Clinical Chemistry

sodium (NA)	creatinine (CREAT)
potassium (K)	SGOT/AST (AST)
chloride (CL)	SGPT/ALT (ALT)
total protein (TPROT)	globulin (GLOB)
albumin (ALB)	alkaline phosphatase (ALP)
calcium (CA)	cholesterol (CHOL)
phosphorus (PO4)	triglycerides (TRIG)
total bilirubin (TBIL)	A/G ratio (A/G)
urea nitrogen (BUN)	glucose (GLU)

Gross pathology: All animals from the main study group were subjected to gross necropsy observation. There were no gross lesions in the necropsy observations.

Organ weights: All organs from the standard tissue list were studied. There were no changes in the organ weights.

Histopathology: Adequate Battery: Yes
Peer review: Yes, EPL consultants;
The histopathological lesions were associated with chronic inflammation of lungs, acute inflammation in liver, cardiomyopathy, bone marrow cell hyperplasia in femur, injection site reactions, extramedullary hematopoesis in spleen, and congestion in kidney.
The peer reviewer pathologist mentioned that the lung lesions consisted of perivascular mononuclear cell infiltrates and hyperplastic alveolar epithelial cells within the alveoli. According to the pathologist, the lesion was formed as a result of intravascular cannulation and infiltration of foreign particles as observed by the presence of hair and skin structures. The reviewer observed that although the incidence of lung lesions in the test article treated animals were higher than those of the controls; the lesions were not dose related.

The cardiomyopathy in the heart was characterized as minimal for severity index in general. The nature of the lesion was described as small focal area in the myocardium where one of the two myocardial fibers were degenerated and surrounded or infiltrated by a small cluster of mononuclear inflammatory cells. Two females after 2 hrs of GPI 15715 infusion showed moderate cardiomyopathy. In these two females multifocal lesions with mononuclear cell infiltration was noted in both left and right ventricle. The incidence of cardiomyopathy was not noted after 4 hrs of continuous infusion w/GPI 15715; however, the severity of the incidence was described as minimal and described as restricted to one or two small focal areas. Because the increase in the severity was not dose related, the effect of test article in the generation of the cardiomyopathy is questionable. Also note that the pathologist mentioned this lesion as a spontaneous, fairly common lesion in rats.

There was an increased incidence of acute inflammation in liver characterized as minimal to mild in severity in the test article treated animals from all dose groups. The acute inflammation in liver was associated with mono and polymorphonuclear cell infiltrates in the sinusoids. Similar changes were noted in the propofol treated animals. The pathologist mentioned that small clusters of inflammatory cells are usually found in the young rats and the acute inflammation observed in the liver might not be test article related.

There was an increased incidence of congestion in the kidney at mid and high dose; no such changes were noted in the control animals. But similar changes were noted in the propofol treated animals.

There was an increased lesion in the injection sites in the test article treated animals at mid and high dose compared to those of the control and propofol treated animals. The lesions were described as chronic active inflammation characterized by severe in nature in most animals. The lesions were consisted of polymorphonuclear cell infiltration in the fibrin strands; the surrounding fibrovascular area was infiltrated with macrophages and multinucleated giant cells. Several cases had a focal area of hemorrhage and were diagnosed as hematoma.

In addition, there was a dose related increased incidence of bone marrow cell hyperplasia in femur and extramedullary hematopoesis in spleen in the test article treated animals. Similar changes were noted in the propofol treated animals.

Summary of histopathological findings:

Parameters	**Saline**	**GPI 15715 47.5 mg/kg**			**Propofol**
	0	**1hr**	**2 hr**	**4 hr**	**4 hr**
Bone marrow cell/ Femur, Hyperplasia	0/10	2/10 2M	5/10 3M , 2F	6/10 4 M, 2F	6/10 3M, 3F
Heart/Cardiomyopathy	4/10 2M, 2F	3/10 2M, 1F	5/10 3M, 2F	5/10 2M, 3F	4/10 3M, 1F
Lung/Chronic inflammation	5/10 2M, 3F	9/10 5M, 4F	8/10 4M, 4F	6/10 4M, 2F	5/10 2M, 3F

Parameters	Saline	GPI 15715 47.5 mg/kg			Propofol
	0	1hr	2 hr	4 hr	4 hr
Injection site/Chronic active inflammation	1/10 1F	0/10 1F	5/10 3M, 2F	4/10 1M, 3F	1/10 1F
Kidney/Congestion	0/10	0/10	1/10 1F	3/10 1M, 2F	2/10 1M, 1F
Liver/Acute inflammation	1/10 1F	2/10 2F	1/10 1M	4/10 3M, 1F	3/10 1M, 2F
Spleen/Extramedullary hematopoesis	4/10 2M, 2F	5/10 4M, 1F	6/10 4M, 2F	7/10 3M, 4F	8/10 3M, 5F

Notes: M: male, F: female; severity index for all lesions were minimal except cardiomyopathy in mid dose which were considered moderate, and injection site lesions which were considered moderate-severe.

Toxicokinetics: The toxicokinetic analysis of GPI 15715 and propofol derived from it was analyzed under GLP condition in the [redacted] (Study # Absorp\DM-00-023; Toxicokinetic Report: GPI 15715 and Propofol: A 14-Day Intravenous Infusion Toxicity Study in Rats). GPI 15715 appeared to convert to propofol following single and repeat dose administration in rat (Sponsor's table # 23, 24, and 25). There was extreme individual variability in the AUC and the Cmax values; therefore interpretation of the data is difficult. There was no apparent accumulation of propofol following the repeat dose administration in rat. The elimination half life ranged between 0.3-1.5 hrs indicating rapid elimination.

b(4)

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Table 22. Summary of Pharmacokinetic Parameters of Propofol in Plasma of Female Rats Following a Four-Hour Continuous Infusion of Propofol (30 mg/kg/hr) on Day 14 (Group 2)

Gender	Dose (mg/kg/hr)	Occasion	Cmax (ng/mL)	Tmax (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	t½ (hr)
Female	30	Day 14	1705.9	2.00	6663	6846	1.0

Calculations based on truncated values

Table 23. Summary of Pharmacokinetic Parameters of GPI 15715, Uncorrected and Corrected GPI 15715-derived Propofol in Plasma of Male and Female Rats During a One-Hour Intravenous Infusion of GPI 15715 (47.5 mg/kg/hr) on Day 14 (Group 3)

Gender	Dose (mg/kg/hr)	Analyte	Occasion	Cmax (ng/mL)	Tmax (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	t½ (hr)
Male	47.5	GPI 15715	Day 14	41301.9	3.00	109137	NC	NC
		Uncorrected Propofol	Day 14	11241.97	3.00	25080	NC	NC
		Corrected Propofol	Day 14	7566.0	3.00	15174	NC	NC
Female	47.5	GPI 15715	Day 14	32622.6	2.00	65045	NC	NC
		Uncorrected Propofol	Day 14	6900.39	2.00	15158	NC	NC
		Corrected Propofol	Day 14	3854.9	2.00	9086	NC	NC

Calculations based on truncated values
NC: Not Calculated because of a limited dataset

Table 24. Summary of Pharmacokinetic Parameters of GPI 15715, Uncorrected and Corrected GPI 15715-derived Propofol in Plasma of Male and Female Rats During a Two-Hours Intravenous Infusion of GPI 15715 (47.5 mg/kg/hr) on Day 14 (Group 4)

Gender	Dose (mg/kg/hr)	Analyte	Occasion	Cmax (ng/mL)	Tmax (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	t½ (hr)
Male	47.5	GPI 15715	Day 14	22201.90	2.00	24978	NC	NC
		Uncorrected Propofol	Day 14	12695.11	2.00	16428	NC	NC
		Corrected Propofol	Day 14	10622.4	2.00	14090	NC	NC
Female	47.5	GPI 15715	Day 14	28836.5	2.00	23764	23797	1.5
		Uncorrected Propofol	Day 14	10265.4	2.08	6767	NC	NC
		Corrected Propofol	Day 14	8903.9	2.08	4578	NC	NC

Calculations based on truncated values
NC: Not Calculated because of a limited dataset

Table 25. Summary of Pharmacokinetic Parameters of GPI 15715, Uncorrected and Corrected GPI 15715-derived Propofol in Plasma of Male and Female Rats During a Four-Hour Intravenous Infusion of GPI 15715 (47.5 mg/kg/hr) on Day 14 (Group 5)

Gender	Dose (mg/kg/hr)	Analyte	Occasion	Cmax (ng/mL)	Tmax (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	t½ (hr)
Male	47.5	GPI 15715	Day 14	66207.90	4.00	84227	84520	0.7
		Uncorrected Propofol	Day 14	17298.57	4.00	26900	27075	1.0
		Corrected Propofol	Day 14	11111.6	4.00	19446	19627	1.0
Female	47.5	GPI 15715	Day 14	20942.20	4.00	64798	64804	0.3
		Uncorrected Propofol	Day 14	9138.50	4.00	29106	29662	1.4
		Corrected Propofol	Day 14	7183.40	4.00	23056	23660	1.5

Calculations based on truncated values

Study title: A Continuous 24-30 Hour Intravenous Infusion Study in Dogs

Key study findings

- The beagle dogs (1/sex/group) were induced to anesthesia by bolus (8-16 mg/kg) intravenous administration of GPI 15715. The animals were then maintained in anesthesia via continuous infusion (68-79 mg/kg/hr).
- The male dog died at approximately 29 hr post loading dose and the female dog died during recovery after 24 hr of continuous infusion.
- The toxicokinetic analysis of GPI 15715 and propofol derived from it showed that the Cmax in the male and the female dogs were approximately 30 and 15 µg/mL respectively and were achieved at 1 min post dosing. The half life of propofol was measured to be 15.9 mins in female.
- The heart rate and MAP decreased in both animals.
- There was a decrease in RBC, hemoglobin, and hematocrit in both of the animals.
- The histopathology findings include mainly lesions in lung, stomach, liver, and kidney.
- The changes in the liver consisted of severe glycogen depletion in both male and female.
- The kidney lesion in both dogs consisted of focal mineral deposits in the medulla (minimal).
- There was also an injection site reaction near the insertion area of the catheters, the nature of the lesions were not described.
- In addition, the lungs of both of the dogs had congestion, edema, hemorrhage, and interstitial cell inflammation with infiltration of alveolar macrophages. In the lungs from the male amorphous eosinophilic material (moderate) was also observed in the intraluminal space in the bronchi.
- The changes in the stomach was observed only in the male and consisted of brown pigmented material (moderate), edema, hemorrhage, congestion, venous thrombi, and necrosis.
- The histopathological lesion was observed in the trachea (slight-severe) of the female only and consisted of edema, hemorrhage, ulcers, and acute/subacute inflammation involving mucosa and muscularis.
- The Sponsor mentioned that the histopathological changes such as injection site and tracheal lesions are related to trauma associated with the catheter insertion and ventilation procedure. The changes observed in the kidney were within the historical control range of the laboratory conducting the study. The changes in the lung, liver, and stomach are related to the anesthetic effect of the test article according to the Sponsor.
- The continuous infusion of GPI 15715 (TDI female: 1640 mg/kg, HED=911 mg/kg, male: 1888 mg/kg, HED=1048 mg/kg) were not tolerated in dogs.

Study number: 3000-15715-00-01n
Volume # and page #: Module 4-eCTD submission; Page#: 1-157

Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 04-08-2000
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715;
Lot # 11228-17-purity 87.36% &
Lot # 11228-26-purity 85.27%
Vehicle: Phosphate buffered saline (pH 8.5)
Methods

Doses: Induction-male 8 mg/kg; female 16 mg/kg
Maintenance-male: 79 mg/kg/hr for 29.38 hr
Maintenance-female: 68.8 mg/kg/hr for 24 hr
Species/strain: Beagle dog
Number/sex/group or time point (main study): 1/sex/group
Route, formulation, volume, and infusion rate: Intravenous bolus and infusion as stated above; GPI 15751 was formulated in vehicle (PBS)
Satellite groups used for toxicokinetics or recovery: None
Age: 1.3 years
Weight: Males-10.3 kg; Females-8.7 kg
Sampling times: The blood samples for the toxicokinetic analyses were collected pre dosing and at 1, 2, 5, 10, 30 mins, and 1, 4, 8, and 24 hrs.
Unique study design or methodology (if any): Two animals (1/sex/group) were first induced to anesthesia via a bolus intravenous administration through catheter implanted at the femur vein. The animals were then maintained for prolonged sedation for 24-30 hrs via infusion using the same route. The animals were put into ventilator for appropriate oxygenation.

Observations times and results

Mortality: The male died during the treatment between 28-29 hrs. The female died during recovery after 4 hrs of continuous infusion with the test article.

Clinical signs: Clinical sign of anesthesia indicated by no response to light touch and arousal at 16 min post dosing, the pedal reflex was present intermittently, muscle tone and palpebral reflex was retained. The animals were ventilated through intratracheal tube from 6-hr of maintenance in this lightly sedated state. Respiratory rate was generally low (12-56 breaths/min). Pulse rate was 121-136 beats/min, and oxygen saturation was 90% or greater. The muzzle and eye of the male dog appeared swollen and red after a 7-hrs of continuous sedation. This animal had watery stool and red exudates. The clinical sign in females after a continuous infusion of 6 hrs were accumulation of fluids in the endotracheal tube, coughing, and blinking of eyes. At this time the oxygen saturation and respiration rate was also dropped in the female. The body temperature in the male and the female dog at pre dosing was 102.9°F and 102.3°F respectively. The body temperature in female decreased to 94.7-102.2°F and the body temperature in the female decreased to 94.7-102.2°F during the dosing period.

Body weight: The body weight was not recorded pre and post dosing. Due to the nature of the study design, evaluation of the body weight assessment was not required.

Food consumption: The food consumption was not recorded pre and post dosing. Due to the nature of the study design, evaluation of the food consumption assessment was not required.

Cardiovascular assessment: ECG and heart rate were evaluated throughout the dosing period. There was no change in the ECG. Measurements of the P-R intervals, QRS intervals, and the QTc were all within the normal limits. However, there was an increase in the heart rate in both male and females as indicated in the Sponsor's table below. There was also an increase in the mean arterial pressure after approximately 20 hrs of the test article infusion as indicated in the following tables from the Sponsor.

	Mean Heart Rate (beats per minute)		
	Pretest	Treatment	% Change
Male	84.2	123.6	+46.8
Female	96.6	134.3	+39.0

	Mean Blood Pressure (mm Hg)					
	Male			Female		
Parameter	Pretest	Treatment	% Change	Pretest	Treatment	% Change
Diastolic	80.8	90.8	+12.3	79.3	96.5	+21.7
Systolic	151.3	153.1	+1.2	135.5	142.6	+5.2
Mean Arterial	103.7	116.1	+12.0	98.6	114.1	+15.7

Hematology and coagulation: The blood was collected pre dose and at the end of the infusion period, the hematological parameters indicated in the study #3000-15715-00-06g were assessed. There changes in the hematology parameters after the test article administration are tabulated in the following table. A decrease in the erythrocytes was noted, however, all of the changes in males and females were not similar. There was a prolongation of the PT:APTT ratio. In male and female increase in the ratio were 1.8 and 1.2 respectively.

Summary of clinical pathology findings:

Hematology			**Clinical chemistry**		
Parameters (% change)	**Male**	**Female**	**Parameters (% change)**	**Male**	**Female**
Hemoglobin	13↓	20↓	Phosphate (Pi)	4.1-fold↑	2.9-fold↑
Hematocrit	26↓	22↓	Calcium	7 ↓	8 ↓
RBC	18↓	21↓	Potassium	19↓	24↓
WBC	45↓	45↑	Alkaline Pase	90↑	55↑
Neutrophil (absolute count)	47↓	69↑	Asparatate aminotransferase	65↑	No Change

Hematology			Clinical chemistry		
Parameters (% change)	**Male**	**Female**	**Parameters (% change)**	**Male**	**Female**
Lymphocytes	44↓	39↓	Bilirubin	2.7-fold↑	No Change
Monocytes	No Change	183↑			
PT: APTT	1.2↑	1.8↑			

Clinical chemistry: The blood was collected pre dose and at the end of the infusion period, the clinical chemistry parameters indicated in the study #3000-15715-00-06g were assessed. The changes in the clinical chemistry parameters are depicted in the clinical pathology table. According to the Sponsor, the changes in the clinical chemistry values are within the historical control range from the laboratory conducting the study.

Urinanalyses: The urine was collected pre dose and at the end of the infusion period, the urinanalyses parameters indicated in the study #3000-15715-00-06g were assessed. There were no changes in the urinanalyses parameters in female; no urine was collected in males.

Gross pathology: The gross lesion include slight to moderate redness in lung, slight to moderate red iliac lymph nodes in both males and females. In the female trachea was also red (slight) and in the male fundic stomach was observed to black (severe).

Organ weights: The weight of the major organs such as heart, liver, lung, kidney, and brain was reported. There were no changes in the organ weight in females; however, in the males the lungs weight was approximately twice of that of the control. There was also an increase in the kidney weight in the male.

Histopathology: Adequate Battery: Yes
Peer review: No
The test article related histopathological changes were noted in several tissues such as lung, stomach, liver, and kidney. The changes in the liver consisted of severe glycogen depletion in both male and female. Also, the kidney in both dogs had focal mineral deposits in the medulla (minimal). There was also an injection site reaction near the insertion area of the catheters. In addition, the lungs of both dogs had congestion, edema, hemorrhage, and interstitial cell inflammation with infiltration of alveolar macrophages. In the lungs from the male amorphous eosinophilic material (moderate) was also observed in the intraluminal space in the bronchi. The changes in the stomach was observed only in the male and consisted of brown pigmented material (moderate), edema, hemorrhage, congestion, venous thrombi, and necrosis. The histopathological lesion was observed in the trachea (slight-severe) of the female only and consisted of edema, hemorrhage, ulcers, and acute/subacute inflammation involving mucosa and muscularis.

The Sponsor mentioned that the histopathological changes such as injection site and catheter lesions were related to trauma associated with the catheter insertion procedure. The changes observed in the kidney were within the historical control range of the laboratory conducting the study, according to the study pathologist. The changes in the lung, liver, trachea, and stomach are related to the anesthetic effect of the test article according to the Sponsor.

Toxicokinetics: The toxicokinetic analysis of GPI 15715 and propofol derived from it was analyzed not under GLP condition in the [redacted] b(4)
(Study # PK-SMP-15715-007a; Toxicokinetic Report: GPI 15715 and Propofol: A Continuous 24-30 Hour Intravenous Infusion Study in Dogs). The Cmax in the male and the female dogs were approximately 30 and 15 µg/mL respectively and were achieved at 1 min post dosing. The half life of propofol was measured to be 15.9 mins in female. The $AUC_{(0\text{-infinity})}$ in female was 216 µg.h./mL. Due to the death of the male $AUC_{(0\text{-infinity})}$ could not be determined.

Animal	Sex	T_{max} (min)	C_{max} (µg/mL)	$AUC_{0\text{-}t}$ (min*µg/mL)	$AUC_{0\text{-}1440}$ (min*µg/mL)	$AUC_{0\text{-}\infty}$ (min*µg/mL)	$T_{1/2elim}$ (min)
9092	M	1.0	29.995	30178.7	30178.7	ND[1]	ND[1]
9356	F	1.0	15.340	20737.9	19097.7	21584.0	15.9

[1]ND = Not determinable, No discernable terminal phase

Study title: GPI 15715 and Propofol: A 3-Day Range Finding Intravenous Infusion Toxicity Study in Dogs

Key study findings

- In this dose range finding study, GPI 15715 was administered to dog (1/sex/group) by bolus (24-38 mg/kg) intravenous injection to induce the anesthesia, the animals were the maintained in anesthesia by intravenous infusion of GPI 15715 (46-64.6 mg/kg/hr/day) for one hr/day for 3 consecutive days.
- Propofol (induction dose 8-10 mg/kg & maintenance dose 24-34 mg/kg/hr/day) was used as a comparator in this study.
- The initiation and recovery time from the anesthesia was observed to be shorter in the propofol treated animals compared to the GPI 15715 treated animals which may be attributed to the time to convert the prodrug to the active metabolite.
- All animals showed a decrease in the blood pressure and heart rate.
- The reviewer noted that the induction and the maintenance dose for the anesthesia for GPI 15715 were theoretical propofol equivalents.

Study number: 3000-15715-00-05n
Volume # and page #: Module 4-eCTD submission; Page#: 1-77
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 04-08-2000

GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715, Lot # 21708-21-3, 84.8% ; Propofol, Lot # 015100, purity 10 mg/mL emulsion containing 100 mg/mL soybean oil, 22.5 mg/mL glycerol, and 12 mg/mL egg lecithin.
Vehicle: Saline

Methods

Doses: GPI 15715/Induction 24-38 mg/kg & maintenance 46-64.6 mg/kg/hr/day
Propofol/Induction 8-10 mg/kg & maintenance 24-34 mg/kg/hr/day
Species/strain: Beagle dog
Number/sex/group or time point (main study): 2/sex/group
Route, formulation, volume, and infusion rate: Intravenous bolus and infusion as stated above; GPI 15715 was formulated in vehicle (saline)
Satellite groups used for toxicokinetics or recovery: None
Age: 7-9 months
Weight: Males 9.7-9.9 kg; Females 6.9-8.8 kg
Sampling times: Toxicokinetic analyses were not done
Unique study design or methodology (if any): This is a dose range finding study to determine the doses for a 14-day repeat dose study. Four animals (2/sex/group) were first induced to anesthesia (either GPI 15715 or propofol) via a bolus intravenous administration through catheter implanted at the cephalic vein. The animals were then maintained in sedation for one hour each day hrs via infusion using the same route (refer to Sponsor's study design table). The animals were provided with the respiratory support during the infusion period. The procedure was repeated for 3 days.

Study design table:

Group	Study Day	Induction Dose[a]			Maintenance Dose[a]			Number of Animals	
		Dose	Vol.	Conc.	Dose	Flow Rate	Conc.	Initial	
		mg/kg	mL/kg	mg/mL	mg/kg/hour	mL/kg/hr	mg/mL	M	F
1 (Propofol)	0	8	0.8	10	24	2.4	10	1	1
	1	8	0.8	10	30	3.0	10		
	2	10	1.0	10	34	3.4	10		
2 (GPI 15715)	0	24	1.2	20	46	2.3	20	1	1
	1	30.4	1.52	20	57	2.85	20		
	2	38	1.9	20	64.6	3.23	20		

[a]The induction dose was adjusted as necessary to allow for the introduction of an endotracheal tube. The maintenance dose was adjusted during the study to maintain the proper plane of anesthesia. Adjustments were made by changing the flow rate.

M = male; F = female; Vol. = volume; Conc. = concentration

The day of dose initiation was defined as Day 0 of the study.

Observations times and results

Mortality: The animals were observed twice a day each day for mortality and morbidity. There was no mortality in this study.

Clinical signs: The adverse clinical signs observed in this study consisted slight to moderate swelling around the eye and the snout in GPI 15715 treated females during infusion at Day 1. The following clinical signs of anesthesia were also monitored. At termination of infusion, the righting reflex and standing recovery was also recorded. The animals were observed to reach a clinical state of anesthesia as indicated by a non responsive arousal state, no muscle movement, less than normal muscle tone, and absence of pedal reflex, absence of palpebral reflex, papillary response to light, and touch.

- Arousal level (no response to the environment, decreased alertness, normal alertness, increased alertness, hyperexcitability)
- Presence or absence of voluntary movements
- Presence or absence of involuntary movements
- Muscle tone (flaccid, less than normal, normal, or rigid)
- Response to light (pupils constrict normally or no response)
- Palpebral reflex (response or no response to touch on eyelids)
- Pedal reflex (response or no response to pressure on foot pads)
- Sensitivity to light touch (response or no response to light touch on the inside of the ear)
- Capillary refill time (CRT)

The recovery from anesthesia was assessed by time required to achieve sternal recumbency (righting reflex) and the time needed for the dog to stand on its own. There were no major adverse reaction associated with the recovery, however, hyperextension of head and neck, forelimb rigidity, and periodic muscle contractions were noted. Note that the recovery time was prolonged in the GPI 15715 treated animals compared to those of the propofol treated animals.

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Text Table 2: Time to Recovery

Group	Study Day	Total Dose[a] (mg/kg)	PED[b] (mg/kg)	Time to Recovery (minutes)		
				Extubation	Righting	Standing
1 (Propofol)						
9539 M	0	44.7	-	9	22	26
	1	45.4	-	8	23	25
	2	44.0	-	25	26	28
9469 F	0	32.0	-	4	8	15
	1	38.0	-	8	12	16
	2	44.0	-	8	12	13
2 (GPI 15715)						
9540 M	0	92.0	48.4	30	47	49
	1	87.4	46	25	43	43
	2	102.6	54	41	43	48
9478 F	0	95.4	50.2	35	35	52
	1	106.6	56.1	29	39	39
	2	102.6	54	15	16	30

[a]Total dose includes the induction dose, the maintenance dose, and any additional bolus doses.
[b]PED = Propofol equivalent dose, based on a GPI 15715/Propofol weight ratio of 1.9.

Body weight: The body weights were recorded each day. There were no test article treated changes in the body weight.

Oxygen saturation in blood: The oxygenation in blood was periodically analyzed and approximately 80-85% oxygen saturation was noted indicating no adverse effect of the test article.

Cardiovascular assessment: There were no changes in the ECG; however, an increased in the heart rate and mean arterial pressure was noted with GPI 15715 as well as propofol administration.

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Text Table 1: Changes in Blood Pressure and Heart Rate

Group	Study Day	Interval[a]	Percent Change from Pretest Values			
			Blood Pressure			
			Systolic (mm Hg)	Diastolic (mm Hg)	MAP (mm Hg)	Heart Rate (bpm)
1 (Propofol)						
9539 M	0	1	+25	+25	+24	+89
		2	+39	+54	+55	+151
	1	1	-	-	-	+90[b]
		2	-	-	-	+97[b]
	2	1	+15	+3	+13	+116
		2	+19	+17	+23	+56
9469 F	0	1	-8	-11	-4	+23
		2	-15	-12	-6	+4
	1	1	+8	+1	+5	+25
		2	-1	-8	-2	+20
	2	1	-	-	-	+28[b]
		2	-	-	-	-1[b]
2 (GPI 15715)						
9540 M	0	1	-29	-47	-41	-13
		2	-19	-32	-26	-6
	1	1	-16	-37	-33	+10
		2	-5	-19	-17	-12
	2	1	-	-	-	+19[b]
		2	-	-	-	-7[b]
9478 F	0	1	-16	-17	-16	+14
		2	-31	-35	-33	+25
	1	1	-	-	-	+32[b]
		2	-	-	-	+29[b]
	2	1	-22	-35	-31	-7
		2	-16	-20	-17	+16

[a]Measurements were performed at two intervals during infusion; the first was generally conducted during the first 15 minutes of infusion and the last was generally conducted during the last 15 minutes of infusion.

[b]Pulse rate, measured using the oximeter, were used for heart rate measurements. All other heart rates were recorded using the [redacted] b(4)

Key: - = No Measurement Taken; M = Male; F = Female

Gross pathology: There were no gross lesions in any of the animals in this study indicating the doses used either for GPI 15715 and propofol were well tolerated.

Study title: GPI 15715 and Propofol: A 14-Day Intravenous Infusion Toxicity Study in Dogs

Key study findings

- The beagle dogs (3/sex/group) were administered with the induction dose (24-38 mg/kg) of the test or the positive control (10 mg/kg) by intravenous bolus injection for 30-90 seconds followed by the intravenous infusion (~65-95 mg/kg/hr/day for GPI 15715 and 34-41 mg/kg/hr /day for propofol), approximately one hour per day for fourteen days.
- There was a comparable increase in the heart rate and MAP in all GPI 15715 and propofol treated animals.
- There was a comparable decrease in the RBC, hemoglobin, and hematocrit all GPI 15715 and propofol treated animals.
- There was evidence of respiratory acidosis in arterial blood samples collected during the final infusion interval with propofol and GPI 15715. The mean bicarbonate level increased by 2.7 mmol/L after propofol treatment in males. Similarly the bicarbonate level increased by 1.7 mmol/L after GPI 15715 treatments in males and females. All these changes in the blood gas analyses indicate that there was depression in the respiratory centers resulting in insufficient alveolar ventilation and CO_2 accumulation.
- The histological findings are restricted mainly to lung, bone marrow cell hyperplasia, injection sites, trachea indicating that these are the major target organ for toxicity.
- The lesion in lungs was associated with increased incidence of the chronic active inflammation of the visceral pleural and interstitium of lungs after GPI 15715 administration. Similar findings were noted after propofol administration in the interstitium of lungs but not in the visceral pleural area.
- Another major finding is the metaplasia in the squamous area of trachea. The incidence and the degree of severity increased slightly in the GPI 15715 and propofol treated animals. The Sponsor did not provide peer review of the histological findings, therefore the nature of the lesions are unknown.
- There was thickening of the injection sites in the GPI 15715 treated animals surrounding the catheter injection area, however, there was no histological lesions associated with it. Trauma associated with the manipulation of the catheter in 3 dogs in the GPI 15715 treated animals exacerbated the severity of the findings.
- GP 15715 increased the incidence of the histological findings and/or increase in the severity of the findings in the lungs and injection site compared to those of the propofol. The TDI for GPI 15715 and propofol were 133 (HED=74) and 51 (HED=28) mg/kg respectively. $AUC_{(0\text{-infinity})}$ for GPI 15715 and propofol were 24 and 20 µg.h/mL respectively.

Study number: 3000-15715-00-06g
Volume # and page #: Module 4-eCTD submission; Page#: 1-391
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 08-08-2000
GLP compliance: Yes
QA reports: Yes

Drug, lot #, and % purity: GPI 15715, Lot # 21708-21-3, 84.8% ; Propofol, Lot # 015100 & 014214, purity 10 mg/mL emulsion containing 100 mg/mL soybean oil, 22.5 mg/mL glycerol, and 12 mg/mL egg lecithin.
Vehicle: Saline

Methods

Doses:

GPI 15715/Induction 24-38 mg/kg & maintenance ~65-95 mg/kg/hr/day
Propofol/Induction 10 mg/kg & maintenance 34-41 mg/kg/hr/day

Species/strain: Beagle dog
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Intravenous bolus and infusion as stated above; GPI 15715 was formulated in vehicle (saline)
Satellite groups used for toxicokinetics or recovery: None
Age: 6-7 months
Weight: Males 7.8-9.9 kg; Females 5.8 -7.1 kg
Sampling times: The blood samples for the toxicokinetic analyses were collected after the first and last dosing pre dose, 2, 4, 6, 10 mins, and 1, 1.33, 1.67, 2, 4, and 6 hrs.
Unique study design or methodology (if any): The animals were administered with the induction dose of the test article or the positive control by intravenous bolus injection for 30-90 seconds followed by the intravenous infusion for approximately one hour per day for fourteen days (refer to Sponsor's study design table). The animals were mechanically ventilated during anesthesia; an endotracheal tube was inserted after the induction dose and/or during the start of the maintenance dose.

EXPERIMENTAL DESIGN

	Induction Dose[a]			Maintenance Dose[a]			Number of Animals									
							Initial		Toxicokinetics[b]		Clinical Pathology[c]		Necropsy		Microscopic Pathology	
	Dose	Volume	Conc.	Dose	Flow Rate	Conc.			Days 0 and 13		Pretest, Day 6 and Day 14		Day 14			
Group	mg/kg	mL/kg	mg/mL	mg/kg/hr	mL/kg/hr	mg/mL	M	F	M	F	M	F	M	F	M	F
1 (saline)	0	0	0	0	3.23-4.7	0	3	3	3	3	3	3	3	3	3	3
2 (Propofol)	10	1	10	34.0-41.1	3.4-4.1	10	3	3	3	3	3	3	3	3	3	3
3 (GPI 15715)	38	1.9	20	64.6-94.6	3.23-4.7	20	3	3	3	3	3	3	3	3	3	3

[a]Doses represent active ingredient. The maintenance dose was adjusted during the study to maintain the proper plane of anesthesia. Adjustments to the dose were made by changing the flow rate. All changes were recorded.

[b]Toxicokinetic samples were collected from each animal in Groups 2 and 3 on Study Day 0 predose; 2, 4, 6, and 10 minutes; and 1, 1.33, 1.67, 2, 3, 4, and 6 hours after the bolus induction dose; and on Day 13 predose; 2, 4, 6, and 10 minutes; and 1, 1.25, 1.5, 1.75, 2, 3, 4, and 6 hours after the bolus induction dose. Blood samples were collected from the control animals (Group 1) on Day 0 and Day 13 prior to the change in flow rate (i.e., the change from 5.0 mL/hr to the rate equivalent to the Group 3 rate), and 1, 3, and 6 hours after the change in infusion rate. In addition, other samples were collected as necessary such that the amount of blood collected from the control animals was equivalent to the amount of blood collected from animals in Groups 2 and 3.

[c]Clinical pathology evaluations comprised hematology, coagulation, clinical chemistry, blood gas analysis and urinalysis. Blood gas analysis was conducted pretest for all groups and the last day of dosing for Groups 2 and 3.

M = male; F = female
The day of dose initiation is Day 0 of the study.

Observations times and results

Mortality: The animals were observed twice daily for mortality and morbidity. There was no mortality in this study.

Physical examination: The animals were examined weekly for evaluating the general condition, skin and fur, eyes, nose, oral cavity, abdomen, and external genitalia as well as evaluation of respiration and observations for any unusual behavior. There were no test article related changes in the physical examinations.

Clinical signs: The clinical signs of anesthesia (parameters indicated in the study # 3000-15715-00-05) were monitored once daily during the treatment period. All animals treated with propofol and GPI 15715 were observed to be in the light sedated state. The following tables reproduced from the Sponsor's submission demonstrate involuntary movements and reflex response to stimuli after the test article administration. The data indicate that other than involuntary movements, incidences of reflexes were similar in propofol and GPI 15715 treated animals.

Summary of clinical sign findings:

Group	Mean Incidence of Finding (Percent of Intervals ± Standard Deviation)				
	Involuntary Movement	Pupil Response	Palpebral Reflex	Pedal Reflex	Response to Light Touch
2 (Propofol)					
Male	9.6 ± 1.9	33.8 ± 11.1	29.8 ± 23.7	21.4 ± 23.4	7.2 ± 3.5
Female	14.3 ± 16.3	36.9 ± 5.5	28.6 ± 18.9	10.7 ± 9.4	7.1 ± 12.4
Total	**11.9 ± 10.7**	**35.3 ± 8.0**	**29.2 ± 19.2**	**16.1 ± 17.0**	**7.2 ± 8.1**
3 (GPI 15715)					
Male	25.0 ± 3.6	38.1 ± 16.9	17.9 ±16.3	7.1 ± 12.4	3.6 ± 6.2
Female	32.1 ± 10.8	42.9 ± 15.6	41.7 ± 12.5	26.2 ± 12.5	19.1 ±8.3
Total	**28.6 ± 8.2**	**40.5 ± 14.8**	**29.8 ± 18.4**	**16.7 ± 15.3**	**11.3 ± 10.7**

The time to achieve righting ability (sternal recumbency) and standing recovery were also observed to be longer in the GPI 15715 treated animals compared to those of the controls as indicated in the following table from the Sponsor.

Appears This Way
On Original

Summary of mean recovery times:

Group	Mean Recovery Times (minutes)	
	Righting	Standing
2 (Propofol)		
Male	18.7 ± 6.7	28.3 ± 3.0
Female	24.3 ± 2.1	29.3 ± 3.2
Total	21.5 ± 5.4	28.8 ± 5.8
3 (GPI 15715)		
Male	40.0 ± 5.6	47.0 ± 7.0
Female	28.0 ± 7.2	36.7 ± 7.4
Total	34.0 ± 8.7	41.8 ± 8.6

Ophthalmological examination: The animals were examined pretest and at the end of the dosing period by indirect ophthalmoscopy to evaluate cornea, anterior chamber, iris, lens, vitreous humor, retina, and optic disc. The eyelids, lacrimal apparatus, and conjunctiva were also examined grossly. There were no ocular abnormalities in the test article treated animals.

Body weight: The body weights were recorded at pre dose, Days 6, 13, and 14. There were no test article treated changes in the body weights.

Food consumption: The food consumption was recorded daily. There were no test article related changes in the food consumption.

Electroencephalogram (EEG) assessment: The EEG (5 leads plus a ground) was recorded from each of the test article treated animals once week. There was a shift in EEG to a **'delta wave' activity associated** with propofol and GPI 15715 induction phase which is consistent with mild to moderate anesthesia. The maintenance was associated with repetitive bursts of low frequency high multitude signals; however, there were no consistent differences in the patterns of EEG changes associated with either propofol or GPI 15715.

Cardiovascular assessment: The ECG (9- lead) was recorded from each test article treated animal weekly. The blood pressure and heart rates were also assessed at the same time. There were no changes in ECG. A statistically significant change in the heart rates was noted in female at Week 2 of the propofol and GPI 15715 administration as indicated in the following table from the Sponsor.

Summary of heart rate changes:

	Mean Heart Rates (bpm)[a] (± Standard Deviation)		
Group	Pretest	Week 1	Week 2
Males			
1 (saline)	105 ± 8	-	111 ± 8
2 (Propofol)	81 ± 22	152 ± 5	146 ± 28
3 (GPI 15715)	110 ± 39	157 ± 21	142 ± 22
Females			
1 (saline)	117 ± 27	-	95 ± 12
2 (Propofol)	106 ± 25	171 ± 4	172 ± 20[b]
3 (GPI 15715)	104 ± 28	149 ± 24	153 ± 18[b]

[a]Heart rates measured from ECG recordings.
[b]Statistically significantly increased relative to the control value ($p<0.01$).

There was an increase in the mean arterial pressure after two weeks of the test article administration in females which might be associated with the increased heart rate. In 2/3 males treated with propofol an increase (26-51%) in blood pressure were noted. No such changes were noted in the males treated with GPI 15715.

Hematology and coagulation: The evaluation of hematology and coagulation was conducted at pre dose, Day 6, and Day 14 from all animals by collecting the blood through jugular venipuncture. Following parameters were analyzed.

b(4)

Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Total leukocyte count
Reticulocyte count
Differential leukocyte count[1]

Other

Erythrocyte morphology (Henry, 1991)

There were no changes in the leukocyte counts except one control male in this study. However, there was a change in the RBC counts, hemoglobin, and hematocrit at Day14 as indicated in the table below. There was an increase in the mean reticulocytes counts. Similar changes were noted also in the propofol treated animals. There were no changes in the coagulation parameters.

Summary of hematological findings:

Hematology/GPI 15715		
Parameters (% change from control)	**Male**	**Female**
GPI 15751		
Hemoglobin	20.5↓	6.4↓
Hematocrit	18.2↓	5.2↓
RBC	21.3↓	7.2↓
Mean corpuscular hemoglobin	3.2↓	2.6↓
Mean reticulocytes count	92.4↑	24.1↑
Propofol		
Hemoglobin	12.8↓	12.8↓
Hematocrit	10.8↓	9.8↓
RBC	14.3↓	10.4↓
Mean corpuscular hemoglobin	2.3↓	No Change
Mean reticulocytes count	57.6↑	68.4↑

Clinical chemistry: The evaluation of clinical chemistry was conducted at pre dose, Day 6, and Day 14 from all animals by collecting the blood through jugular venipunctutre. Following parameters were analyzed. There were no test article related changes in the clinical chemistry parameters.

b(4)

Aspartate aminotransferase (*Kinetic - Modified IFCC Technique*)
Alanine aminotransferase (*Kinetic - Modified IFCC Technique*)
Alkaline phosphatase (*Kinetic – Modified AMP Buffer*)
Blood urea nitrogen (*Kinetic - Modified Urease*)
Creatinine (*Kinetic -Modified Jaffe Method*)
Glucose (*Hexokinase Method*)
Cholesterol (*Enzymatic – Modified Trinder Method*)
Triglycerides (*GPO Triglyceride-lipase Method*)
Total protein (*Biuret Technique*)
Albumin (*Bromocresol Green Method*)
Total bilirubin (*Modified Wahlefield et al.*)
Sodium (*Ion Selective Electrode*)
Potassium (*Ion Selective Electrode*)
Chloride (*Ion Selective Electrode*)
Total Calcium (*Cresolphthalein Complexone Method*)
Inorganic phosphorus (*Phosphomolybdate - UV Method*)

Other

Globulin (*calculated value; total protein - albumin*)
Albumin/globulin ratio (*calculated value; albumin ÷ globulin*)
Ionized Calcium[2]

Blood gas analysis: The evaluation of the blood gases were conducted at pre dose and Day 14 from all animals by collecting the blood from femur vein. Following parameters were analyzed.

[redacted] b(4)

pH
Partial pressure of CO_2
Partial pressure of O_2

Other

Bicarbonate (calculated value)
Total CO_2 (calculated value)
Actual base excess (calculated value)

There was evidence of respiratory acidosis in arterial blood samples collected during the final infusion interval with propofol and GPI 15751. Mean pH values were decreased to approximately 0.1. The pH values were slightly below the normal range (7.35-7.45) for both males and females in all drug treated animals. The partial pressure of CO_2 increased after the propofol (17.9 mmHg for males and 9.0 mmHg for females) and GPI 15751 treatments (13.6 mmHg for males and 15.0 mmHg for females). The mean bicarbonate level increased by 2.7 mmol/L after propofol treatment in males. Similarly the bicarbonate level increased by 1.7 mmol/L after GPI 15715 treatments in males and females.

All these changes in the blood gas analyses indicate that there was depression in the respiratory centers resulting in insufficient alveolar ventilation and CO_2 accumulation.

Urinanalyses: The urinanalyses were performed from the sample collected prior to necropsy. Following parameters were analyzed. There was no evidence of a test article treated changes in any of the parameters studied within the urinalyses.

[redacted] b(4)

Nitrites
Protein
Glucose
Ketones
pH
Bilirubin

Urobilinogen

Protein results of 100 mg/dL or greater were verified using a three percent sulfosalicylic acid test. Positive bilirubin results were confirmed via [redacted] b(4)

Sodium ***(Ion Selective Electrode)***
Potassium ***(Ion Selective Electrode)***
Chloride ***(Ion Selective Electrode)***
Calcium ***(Cresolphthalein Complexone Method)***
Inorganic phosphorus ***(Phosphomolybdate-UV Method)***

Other

Appearance
Specific gravity [redacted] b(4)

Gross pathology: Complete macroscopic evaluation was performed on all animals. The major findings include thickening of vena cava surrounding the catheter insertion area and enlargement of the iliac lymph nodes.

Organ weights: The organ weights reported from this study is listed in the histopathology table; the major organs were weighed. There were no biologically significance changes in the organ weights. The only change observed was a slight statistically significant increase in the kidney weight compared to control (0.5 g in control vs-0.6 g in GPI 15715 treated animals)

Histopathology: Adequate Battery: Yes
Peer review: No
The histopathological examinations were conducted in all tissues from the standard tissue list. The histological findings are restricted mainly to lung, bone marrow cell hyperplasia, injection sites, and trachea indicating that these are the major target organs for toxicity. The lesion in lungs was associated with increased incidence of the chronic active inflammation of the visceral pleural and interstitium of lungs after GPI 15715 administration. Similar findings were noted after propofol administration in the interstitium of lungs but not in the visceral pleural area. Another major finding is the metaplasia in the squamous area of trachea. The incidence and the degree of severity increased slightly in the GPI 15715 treated animals. The Sponsor did not provide peer review of the histological findings, therefore the nature of the lesions are unknown. There was thickening of the injection sites in the GPI 15715 treated animals surrounding the catheter injection area, however, there was no histological lesions associated with it. Trauma associated with the manipulation of the catheter in 3 dogs in the GPI 15715 treated animals exacerbated the severity of the findings. All of the histopathological lesions associated with an increase in incidence and/or increase in the severity of the findings compared to those control are considered test article related by the reviewer. The histological findings in lungs were attributed to the anesthetic property of the test articles; bone marrow cell hyperplasia might be a consequence of the erythrocyte depletion due to the high volume of the test article administered during the infusion. The

squamous cell metaplasia in the trachea are believed to be associated with the cannulation as the lesions were also noted in the control animals, however, higher incidence of the lesions in the test article treated animals indicate exacerbation of the lesions in the presence of the test article.

Summary of gross lesions & histopathological findings:

Parameters	Saline	GPI 15715	Propofol
Bone marrow cell/ Femur, Hyperplasia	**0/6**	**1/6 1M, minimal**	**1/6 1F, minimal**
Lung/Discolored	**0/6**	**3/6 1M, 2F, minimal**	**2/6 2F, minimal**
Lung/Visceral & Pleural/ Chronic active Inflammation	**0/6**	**1/6 1F, minimal**	**0/6**
Lung/Visceral & Pleural /Fibrosis	**0/6**	**1/6 1F, minimal**	**0/6**
Lung/Interstitium/ Chronic active Inflammation	**0/6**	**2/6 1F, minimal 1M, moderate**	**3/6 1M, 2F, minimal**
Injection site/ Catheter insertion area swollen/thickened	**1/6 1M, minimal**	**3/6 1M, 2F**	**1/6**
Tracheal mucosa/Squamous cell metaplasia	**5/6 2M, minimal 2F, minimal 1F, slight**	**6/6 1M, minimal 2M, 3 F, slight**	**6/6 1M, 1F, minimal 2M, 2F, slight**

Toxicokinetics: The toxicokinetic analysis of GPI 15715 and propofol derived from it was analyzed under GLP condition at [redacted] (Study # Absorp\DM-00-022; Toxicokinetic Report: GPI 15715 and Propofol: A 14-Day Intravenous Infusion Toxicity Study in Dogs). GPI 15715 appeared to convert to propofol following single and repeat dose administration in dog. The Cmax and $AUC_{0\text{-infinity}}$ of propofol after 51 mg/kg of the propofol administration at Day 14 were approximately 14 µg/mL and 20 µg.h/mL respectively indicating no accumulation. Similarly, the Cmax and $AUC_{0\text{-infinity}}$ of propofol after 133 mg/kg of the GPI 15715 administration at Day 14 were approximately 20 µg/mL and 24 µg.h/mL respectively. There were no measurable differences in the exposures at Days 0 and 18 indicating no accumulation. The elimination of propofol was rapid as indicated a mean half life value of approximately 1.55 hrs at Day 13. There were no accumulations of either of the compounds. No gender differences were noted, $AUC_{0\text{-infinity}}$ in males and females ranged between 21-28 µg.h/mL at Day 13 and 19-21 µg.h/mL at Day 0.

b(4)

Table 17. Summary of Toxicokinetic Parameters of Propofol in Plasma of Male and Female Dogs During a 14-Day Daily Intravenous Infusion of Propofol

Gender	Dose* (mg/kg/day)	Occasion	Cmax (ng/ml)	Tmax (hr)	AUC_{0-t} (ng*hr/ml)	$AUC_{0-\infty}$ (ng*hr/ml)	t½ (hr)	CL (mL/hr)
Male	44	Day 0	13106.447	0.353	16366.2	17131.5	2.01	2683.324
			(1631.7719)	(0.5600)	(3612.21	(4054.27)	(0.549)	(729.4322)
	44	Day 13	16000.543	0.400	19202.4	19904.0	2.28	2292.498
			(2957.38267)	(0.5243)	(4279.94)	(4319.37)	(0.706)	(566.3450)
Female	44	Day 0	15022.956	0.677	17554.0	17838.6	1.16	2480.167
			(2320.1128)	(0.5600)	(1637.34)	(1645.51)	(0.280)	(221.3888)
	44	Day 13	16190.414	0.353	19272.4	20278.5	2.81	2215.239
			(4147.0386)	(0.5600)	(4171.52)	(3733.87)	(1.801)	(370.4303)

*: Total dose administered (induction + maintenance)
Calculations based on truncated values
Values in parentheses represent standard deviation

Table 18. Summary of Toxicokinetic Parameters of GPI 15715 in Plasma of Male and Female Dogs During a 14-Day Daily Intravenous Infusion of GPI 15715

Gender	Dose* (mg/kg/day)	Occasion	Cmax (ng/ml)	Tmax (hr)	AUC_{0-t} (ng*hr/ml)	$AUC_{0-\infty}$ (ng*hr/ml)	t½ (hr)	CL (mL/hr)
Male	102.6	Day 0	292726.90	0.030	138567.1	138616.2	0.73	773.167
			(39078.928)	(0.0000)	(32714.62)	(32728.27)	(0.100)	(209.8181)
	102.6	Day 13	250640.93	0.030	95363.9	105496.1	0.62	1025.919
			(34589.246)	(0.0000)	(29764.59)	(34028.69)	(0.182)	(330.9192)
Female	102.6	Day 0	246096.10	0.030	102314.1	114804.9	0.56	899.350
			(28498.596)	(0.0000)	(23445.09)	(12875.25)	(0.042)	(100.8617)
	102.6	Day 13	221510.14	0.030	85680.1	85731.1	2.51	1398.763
			(65612.507)	(0.0000)	(35897.41)	(35856.75)	(3.543)	(732.5843)

*: Total dose administered (induction + maintenance)
Calculations based on truncated values
Values in parentheses represent standard deviation

Table 20. Summary of Toxicokinetic Parameters of Corrected Propofol in Plasma of Male and Female Dogs During a 14-Day Daily Intravenous Infusion of GPI 15715

Gender	Dose* (mg/kg/day)	Occasion	Cmax (ng/ml)	Tmax (hr)	AUC_{0-t} (ng*hr/ml)	$AUC_{0-\infty}$ (ng*hr/ml)	t½ (hr)
Male	102.6	Day 0	22627.34	0.377	21044.2	21614.0	1.25
			(20347.256)	(0.5410)	(7679.39)	(7354.64)	(0.162)
	102.6	Day 13	25475.36	0.08	27967.1	28858.9	1.55
			(9021.941)	(0.0173)	(7378.37)	(7712.65)	(0.812)
Female	102.6	Day 0	18050.29	0.080	19871.9	20307.1	1.57
			(8260.452)	(0.0173)	(4840.47)	(4785.02)	(0.537)
	102.6	Day 13	18408.05	0.390	20993.7	21477.9	1.66
			(5362.645)	(0.5285)	(6318.32)	(6199.03)	(0.275)

*: Total dose administered (induction + maintenance)
Calculations based on truncated values
Values in parentheses represent standard deviation

Study title: GPI 15715 and Propofol: A 48-Hour Intravenous Infusion Toxicity Study in Cynomolgus Monkeys

Key study findings

- The cynomolgus monkeys (3/sex/group) were induced to anesthesia by bolus (20 mg/kg) intravenous administration of either GPI 15715 or propofol (10 mg/kg). The animals were then maintained in anesthesia via continuous infusion of either GPI 15715 (~57 mg/kg/hr) or propofol (~30 mg/kg/hr).

- There were 4 unscheduled deaths in this study. The propofol infusion in males resulted in 2/3 (animals #s 1265 and 1266) deaths between 36-42 hrs. Two animals (one male and one female) also died after GPI 15715 administrations. The male (animal #2266) died during recovery at 48 hrs of the study drug administration) and the female (animal #2767 died at 22.8 hrs) was sacrificed due to humane reason related to the severe subcutaneous edema.
- The death in males appeared to be related to the duration of infusion and/or the total dose received. Each of these animals showed ECG abnormalities, cardiac myodegeneration, edema, and hemorrhage. The female was sacrificed due to humane reason related to the severe subcutaneous edema.
- The toxicokinetic analyses were designed to compare the exposures of propofol after the IV administration of propofol itself and the prodrug (GPI 15715). The exposures of propofol as described by AUC were comparable in all animals (230-330 μg.h/mL). The Cmax was higher in the propofol (23 μg/mL) treated animals compared to that of the GPI 15751 (18 μg/mL) treated animals. The formate exposures were similar in all groups (2437-2680 μg.h/mL).
- There were evidence of blood loss in 3/6 animals from the propofol treated group. Similar incidence of blood loss was noted in the GPI 15715 treated animals. Two females from the propofol group vomited blood. In two females from the GPI 15715 treated group blood was noted in the endotracheal tube. One male from the GPI 15715 treated group had a black tarry stool. In addition, edema, swollen eyelids, swollen tongues were seen occasionally in both propofol and GPI 15715 treated animal.
- There was a decrease in heart rate and blood pressure in all animals treated with GPI 15715 and propofol.
- There was a consistent decrease in the hematocrit level during infusion which was not recovered at the time of necropsy in all animals treated with GPI 15715 and propofol.
- There was a decrease in pH values apparently related to an accumulation of CO_2 and a compensatory increase in HCO_3. The CO_2 accumulation was attributed to insufficient alveolar ventilation as a result of the depression of the respiratory centers in the brain by the anesthetics. The increase in pH indicates an acidotic condition in the animals. Similar changes were not always observed in the GPI 15715 treated animals.
- The histological findings were restricted mainly to striated muscles such as heart and skeletal muscle. There were test article related histopathological changes also in the spleen and the skin. Histological lesion in heart consisted of atrial and left ventricular subendocardial myocardial degeneration accompanied by neutrophilic infiltrates corroborating cardiac insufficiency. Some animals surviving to scheduled necropsy had increased neutrophilic infiltrates with myocardiocytes having large nuclei (karyomegaly) with prominent nucleoli.
- The histological changes in the skeletal muscles were associated with fibrovascular stroma (perimysium) separating bundles of myofibers accompanied with acute myodegeneration due to neutrophil infiltration. Some animals surviving to scheduled necropsy had distinctive histological changes comprising

myofibers loss and conspicuous myofibers regeneration lining the perimysial framework.

- The histological changes in the spleen consisted of lymphcytosis in the splenic white pulp. This lesion was observed in all treated animals, the lesions were, however, more pronounced among animals that died or were euthanized around the infusion period. Similar changes were observed in animals subjected to stress related endogenous corticosteroid release. The spleen lesions are therefore considered as a treatment related secondary effect.
- The histological changes in the skin were observed in 1/3 females treated with GPI 15715. This animal had squamous cell hyperplasia, necrosis of the epidermal layer, neutrophilic infiltration with deep mural arteritis, and bacterial contamination. The biological relevance of this isolated finding is not known.
- Most of the treated animals also had tracheal lesions including submucosal inflammation and epithelial loss associated with metaplasia. These changes were attributed to mild local trauma associated with intubation.
- The severity index and incidence of histological lesions were higher in myocardial tissue and skin in the GPI 15715 treated animals compared to those of propofol treated animals.
- The continuous infusion of GPI 15715 (TDI=1388 mg/kg; $AUC_{0\text{-infinity}}$ = 280 µg.h/mL; HED=448 mg/kg) and propofol (TDI=730 mg/kg; $AUC_{0\text{-infinity}}$ = 280 µg.h/mL; HED=235 mg/kg) was not well tolerated in the monkeys.

Study number: 3000-15715-01-02g
Volume # and page #: Module 4-eCTD submission; Page#: 1-884
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 03-20-2001
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, Lot # 12164-GI-00-001, 93.3% ; Propofol [redacted], Lot # 01A312 & 01A303, purity 10 mg/mL emulsion containing 100 mg/mL soybean oil, 22.5 mg/mL glycerol, and 12 mg/mL egg lecithin. b(4)
Vehicle: Saline

Methods

Doses:
GPI 15715: Induction 20 mg/kg & maintenance 57 mg/kg/hr/day
Propofol: Induction 10 mg/kg & maintenance 30 mg/kg/hr /day
Species/strain: *Macaca fascicularis*
Number/sex/group or time point (main study): 3/sex/group
Route and formulation: Intravenous bolus and infusion as stated above; GPI 15715 was formulated in vehicle (saline); propofol was used as received from supplier.
Satellite groups used for toxicokinetics or recovery: None
Age: 3.5-5.5 years

Weight: Males 3.0-3.8 kg; Females 2.2 -3.0 kg
Sampling times: The blood samples for the toxicokinetic analyses were collected from post onset of infusion and post termination of infusion. The detail of the blood sampling time is reproduced in **Sponsor's table in the observation and** results section.
Unique study design or methodology (if any): The animals were administered with the induction dose of the test article or positive control by intravenous bolus injection for 30-90 seconds via saphenous or cephalic vein. This was followed by the intravenous infusion with the maintenance dose originally scheduled to be delivered for 48 hours. The study was conducted in 3 sessions. In the first session, 4 males (2 from the propofol treatment group and 2 from the GPI 15715 treatment group) were scheduled to be infused with the maintenance dose for 48 hrs, however, ¾ animals died in the first session. Therefore the duration of infusion was reduced to 24 hrs. All animals were mechanically ventilated during anesthesia; an endotracheal tube was inserted after the induction dose and/or during the start of the maintenance dose. In addition, saline was infused continuously in animals after the first session to maintain blood pressure. Prior to the initiation of the study; the animals were implanted with telemetry devices for cardiovascular assessments. Following is **the Sponsor's study design table.**

Study design table:

	Induction Dose[a]			Maintenance Dose[a]			Number of Animals									
	Dose	Volume	Conc.	Dose	Flow Rate	Conc.	Initial		Toxicokinetics[b]		Clinical Pathology[c]		Necropsy[d]		Microscopic Pathology	
Group	mg/kg	mL/kg	mg/mL	mg/kg/hr	mL/kg/hr	mg/mL	M	F	M	F	M	F	M	F	M	F
1 (Propofol)	10	1.0	10	30	3.0	10	3	3	3	3	3	3	3	3	3	3
2 (GPI 15715)	38	1.9	20	57	2.85	20	3	3	3	3	3	3	3	3	3	3

[a]Doses were adjusted during the study to maintain the proper plane of anesthesia and physiological function. Adjustments to the maintenance dose were made by changing the flow rate. All changes were recorded.
[b]Blood samples for toxicokinetics were collected as indicated in Section 2.22 (Page 39).
[c]Clinical pathology evaluations are comprised of hematology, coagulation, clinical chemistry, and urinalysis. Blood samples for clinical pathology were collected pretest, prior to the end of infusion, and prior to necropsy. Samples for blood gas analysis were collected from each animal under anesthesia at approximately 8 or 24 hours after the onset of infusion and during the last hour of infusion.
[d]Animals were euthanized and a complete necropsy was performed 5 to 6 days following termination of infusion. A complete necropsy was also performed for any animal that died during the study.
M = male; F = female
The day of dose initiation is Day 0 of the study.

Observations times and results

Mortality: The animals were continuously observed in their cages for mortality and general condition. There were 4 unscheduled deaths in this study. The propofol infusion in males resulted in 2/3 (animals #s 1265 and 1266) deaths between 36-42 hrs. One male

(animal #2266) died during recovery at 48 hrs of the study drug administration and one female (animal # 2767) died at 22.8 hrs. The death in males appeared to be related to the duration of infusion and/or the total dose received. Each of these animals showed ECG abnormalities, cardiac myodegeneration, edema, and hemorrhage. The female was sacrificed due to humane reasons related to the severe subcutaneous edema. The **Sponsor's table reproduced below showed to**tal dose administered to the individual animals. Considerable variations between animals were noted as regards to the duration of infusion resulting in differences in the total daily intake of the test article and the positive control.

Text Table 1

Group	Animal No.	Duration of Infusion (hr)	Mean Maintenance Dose (mg/kg/hr)		Total Dose (mg/kg)	
			Dose	Propofol Equivalent Dose[a]	Dose	Propofol Equivalent Dose[a]
1 Propofol	1265 M	36.7	22.3	-	819.4	-
	1266 M	42.3	25.8	-	1091.6	-
	1267 M	23.7	16.7	-	396.3	-
	1765 F	7.5	30.3	-	226.8	-
	1766 F	6.0	29.9	-	180.0	-
	1767 F	6.4	27.2	-	172.4	-
2 GPI 15715	2265 M	48	40.2	21.2	1929.7	1015.6
	2266 M	48	54.4	28.6	2608.7	1373.0
	2267 M	24	37.8	19.9	907.0	477.4
	2765 F	3.8	31.7	16.7	120.9	63.6
	2766 F	22.7	43.5	22.9	985.1	518.5
	2767 F	22.8	36.9	19.4	840.3	442.3

[a]Propofol equivalent doses were based on a molecular weight ratio of GPI 15715 to Propofol equal to 1.9.
M = male; F = female

Physical examination: The animals were examined pretest and at necropsy for evaluating the general condition, skin and fur, eyes, nose, oral cavity, abdomen, and external genitalia as well as evaluation of respiration and observations for any unusual behavior. There was evidence of blood loss in 3/6 animals treated with propofol. Similar incidence of blood loss was noted in the GPI 15715 treated animals. Two females from the propofol group vomited blood. In two females from the GPI 15715 treated group blood was noted in the endotracheal tube. One male from the GPI 15715 treated group had a black tarry stool. In addition, edema, swollen eyelids, swollen tongues were seen occasionally in both propofol and GPI 15715 treated animals.

Clinical signs: The clinical signs of anesthesia (parameters indicated in the study # 3000-15715-00-05n) were monitored once during the first hour of infusion and approximately 4-6 hrs intervals thereafter. As indicated in **the Sponsor's table #s 2 and 3,** all of the animals appeared to achieve moderate to deep sedation during the infusion period. The animals were unresponsive to external stimuli indicated by no response to light reflex, palpebral response, papillary response, and pedal reflex. There were no voluntary or involuntary movements. Muscle tone was described as flaccid. However, there were episodes of awakening during the last half of the infusion period. This resulted in an increase in the bolus and infusion doses.

Recovery from anesthesia was measured by mean times to achieve righting reflex and standing capabilities. The mean recovery time in the GPI 15715 treated animals was higher (2-3 folds) compared to propofol treated animals (refer to Sponsor's table 4).

Summary of clinical sign findings:

Text Table 2

Animal No.	Total Dose mg/kg	Total PED* mg/kg	No. of Observation/Number of Observation Intervals			
			Arousal Level	Voluntary Movements	Involuntary Movements	Muscle Tone
			Unconscious	Not Present	Not Present	Flaccid
Propofol						
1767 F	172.4	-	2/3	2/3	2/3	1/3
1766 F	180.0	-	2/2	2/3	0/3	0/2
1765 F	226.8	-	3/3	3/3	3/3	0/3
1267 M	396.3	-	6/9	9/9	7/9	2/9
1265 M	819.4	-	12/13	11/13	12/13	10/13
1266 M	1091.6	-	16/17	17/17	14/17	14/17
GPI 15715						
2765 F	120.9	63.6	2/2	2/2	2/2	0/2
2767 F	840.3	442.3	6/6	6/6	6/6	2/6
2267 M	907.0	477.4	6/7	7/7	6/7	2/7
2766 F	985.1	518.5	5/7	6/7	6/7	1/7
2265 M	1929.7	1015.6	16/18	18/18	16/18	12/18
2266 M	2608.7	1373.0	13/14	14/14	8/14	3/14

*Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

Text Table 3

Animal No.	Total Dose mg/kg	Total PED* mg/kg	No. of Observations/No. of Observation Intervals			
			Palpebral Response	Pedal Reflex	Pupillary Response	Sensitivity to Light Touch
			No response	No response	Do not constrict	No response
Propofol						
1767 F	172.4	-	2/3	2/3	0/3	2/3
1766 F	180.0	-	2/3	2/2	2/2	2/2
1765 F	226.8	-	3/3	3/3	2/3	3/3
1267 M	396.3	-	6/9	8/9	4/9	8/9
1265 M	819.4	-	12/13	13/13	9/13	13/13
1266 M	1091.6	-	15/17	17/17	13/17	16/17
GPI 15715						
2765 F	120.9	63.6	2/2	2/2	1/1	2/2
2767 F	840.3	442.3	6/6	6/6	2/6	6/6
2267 M	907.0	477.4	7/7	5/7	5/7	7/7
2766 F	985.1	518.5	6/7	5/7	5/7	6/7
2265 M	1929.7	1015.6	14/18	16/18	8/18	17/18
2266 M	2608.7	1373.0	13/14	12/14	9/14	14/14

*Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

Summary of recovery time findings:

Text Table 4

Animal No.	Total Dose mg/kg	Total PED* mg/kg	Recovery Times (minutes)	
			Righting Recovery	Standing Recovery
Propofol				
1767 F	172.4	-	25	31
1766 F	180.0	-	22	37
1765 F	226.8	-	14	16
1267 M	396.3	-	Not documented	Not documented
1265 M	819.4	-	Animal died	Animal died
1266 M	1091.6	-	Animal died	Animal died
GPI 15715				
2765 F	120.9	63.6	86	121
2767 F	840.3	442.3	49	94
2267 M	907.0	477.4	25	43
2766 F	985.1	518.5	42	90
2265 M	1929.7	1015.6	42	49
2266 M	2608.7	1373.0	Animal sacrificed	Animal sacrificed

*Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9.

Ophthalmological examination: The animals were examined pretest and at the end of the dosing period by indirect ophthalmoscopy to evaluate cornea, anterior chamber, iris, lens, vitreous humor, retina, and optic disc. The eyelids, lacrimal apparatus, and conjunctiva were also examined grossly. There were no ocular abnormalities in the test article treated animals.

Measurement of % O_2 saturation: Measurements were recorded at approximately 6 hrs intervals during infusion. All animals were mechanically ventilated; therefore oxygen saturation was > 90% in most case. However, in one propofol treated animal oxygen saturation was 61%, this animal eventually died.

Body weight: Body weights were recorded at pre dose and at termination. There were no test article related changes in the body weights.

Food consumption: Food consumption was recorded pretest and was not recorded during anesthesia. There was obviously a decrease in food consumption during the prolonged sedation.

Cardiovascular assessment: Cardiovascular data was collected via manual electrocardiography and implanted telemetry devices. Blood pressure and heart rates were also assessed at the same time. There were changes in ECG consisting of the changes in the QRS and ventricular extrasystoles in all animals after propofol and GPI **15715 infusion (refer to Sponsor's table # 5).** There were no relationships to total dose or a pattern in the time of the onset of these changes. However, the ECG changes were associated with the microscopic findings of myodegeneration. There was no apparent change in the heart rate. The mean arterial pressure decreased during the infusion period but recovered as indicated in **the Sponsor's table # 6.**

Summary MAP changes:

Text Table 6

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Approximate Mean Blood Pressure Range (mm Hg)		
			Pretest	Infusion	Recovery
Propofol					
1767 F	172.4	-	75-100	45-75[b]	NS[c]
1766 F	180.0	-	70-100	60-85	75-100
1765 F	226.8	-	75-100	60-85	80-105
1267 M	396.3	-	80-115	70-90	85-115
1265 M	819.4	-	90-120	60-100	NS
1266 M	1091.6	-	90-115	60-90	60-140
GPI 15715					
2765 F	120.9	63.6	85-140	50-100	90-140
2767 F	840.3	442.3	90-120	70-95	100-140
2267 M	907.0	477.4	80-110	60-90	90-120
2766 F	985.1	518.5	90-120	60-80	90-130
2265 M	1929.7	1015.6	70-90	65-85	85-105
2266 M	2608.7	1373.0	80-120	60-90	60-80

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Blood pressure was manually recorded using [redacted] monitor because of loss of the telemetry signal. b(4)

[c]NS = No telemetry signal

Hematology and coagulation: The blood sample for hematology and coagulation studies were obtained via femoral venipunctutre from all animals pretest, during the last hour of infusion, and prior to necropsy. The hematology and coagulation parameters evaluated are listed with the study # 3000-15715-00-06g. There were no consistent pattern of changes in the leukocytes and neutrophil counts in propofol and GPI 15715 treated animals as indicated in the Sponsor's table # 7 & 8. There was, however, a consistent decrease in the hematocrit level during infusion which was not recovered at the time of necropsy. The PT and the APTT level increased during infusion, but generally recovered prior to necropsy, however, in one animal #1266 which died shortly after the termination of infusion, a prolongation of the infusion time by approximately 43 seconds were noted.

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Summary of hematological changes:

Text Table 7

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Changes in Hematology Parameters, as Compared to Pretest Values					
			Hematocrit		Reticulocytes		Platelets	
			Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]
Propofol								
1767 F	172.4	-	-13%	-26%	-60%	+210%	-23%	+12%
1766 F	180.0	-	-24%	-14%	-930%	+180%	-28%	+35%
1765 F	226.8	-	-3%	-5%	-73%	+65%	-11%	+29%
1267 M	396.3	-	-25%	-17%	+45%	+300%	-25%	+36%
1265 M	819.4	-	NS	NS	NS	NS	NS	NS
1266 M	1091.6	-	-7%	NS	+140%	NS	-9%	NS
GPI 15715								
2765 F	120.9	63.6	-10%	-21%	+27%	+330%	-34%	+1%
2767 F	840.3	442.3	-26%	-41%	-19%	+220%	-53%	-30%
2267 M	907.0	477.4	-10%	-13%	-48%	+300%	-16%	+36%
2766 F	985.1	518.5	-18%	-19%	-36%	+400%	-28%	+52%
2265 M	1929.7	1015.6	-31%	-21%	-33%	+180%	-24%	+70%
2266 M	2608.7	1373.0	-31%	NS	-29%	NS	-31%	NS

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Measured during the last hour of infusion.

[c]Measured prior to necropsy.

M = male; F = female; NS = no sample

Text Table 8

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Changes in Hematology Parameters, as Compared to Pretest Values					
			Total Leukocyte Count		Neutrophil Count		Lymphocyte Count	
			Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]
Propofol								
1767 F	172.4	-	-23%	-23%	-3%	-47%	-35%	-8%
1766 F	180.0	-	-18%	+29%	+51%	+2%	-52%	+38%
1765 F	226.8	-	+2%	-11%	+0.5%	-38%	-8%	+20%
1267 M	396.3	-	-21%	-14%	+56%	+17%	-66%	-27%
1265 M	819.4	-	NS	NS	NS	NS	NS	NS
1266 M	1091.6	-	+55%	NS	+190%	NS	-78%	NS
GPI 15715								
2765 F	120.9	63.6	-11%	-23%	+14%	-34%	-60%	-3%
2767 F	840.3	442.3	-13%	+47%	-9%	+50%	-21%	+15%
2267 M	907.0	477.4	+5%	+61%	+160%	+280%	-39%	+1%
2766 F	985.1	518.5	-20%	+22%	-30%	+36%	-16%	-4%
2265 M	1929.7	1015.6	-64%	-28%	-62%	-46%	-69%	+3%
2266 M	2608.7	1373.0	-70%	NS	-49%	NS	-86%	NS

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Measured during the last hour of infusion.

[c]Measured prior to necropsy.

M = male; F = female; NS = no sample

Clinical chemistry: The blood samples for clinical chemistry studies were obtained via femoral venipunctutre from all animals pretest, during the last hour of infusion, and prior to necropsy. The clinical chemistry parameters evaluated are listed with the study # **3000-15715-00-06g. As shown in the Sponsor's table #9 below, serum albumin and** globulin decreased for all animals during the infusion period. The total protein values increased at recovery. The decrease in the serum protein levels might be associated with

the blood loss during infusion. There was a decrease in the serum calcium level in all GPI 15715 and propofol treated animals most probably due to the decrease in the total serum protein level. It is well known that the serum calcium is bound normally to the serum protein. Ionized calcium level decreased in the propofol treated animals but not in the GPI 15715 treated animal. Another consistent finding was a clear increase in the serum triglyceride levels for both propofol and GPI 15715 treated animals. This increase was higher in the propofol treated animals compared to the GPI 15715 treated animals most probably due to the lipophilic nature of the vehicle of propofol. However, increases in the triglycerides by the water soluble prodrug for propofol suggest a stress related endogenous corticosteroid induced effect. Other changes in the clinical chemistry parameters include a decrease (18-45% for the propofol treated animals and 23-49% for the GPI 15715 treated animals) in the alkaline phosphatase level at the end of the infusion period which, however, recovered prior to necropsy. An increase in alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen was also noted at the end of the infusion period in some animals from the propofol and the GPI 15715 treated **group as indicated in the Sponsor's table** #11. Although no microscopic changes were observed in liver in the animals with high ALT and AST level, hemorrhage, edema, degeneration was seen microscopically in the skeletal muscles in these animals. Therefore, increase in AST and ALT may reflect damage in skeletal muscles. The increase in BUN was not associated with any histological changes in the kidney, however, may indicate decrease perfusion efficiency as a result of the debilitated condition of the animals. Interestingly, in some of the propofol treated animals where emesis was observed, a change in the sodium and chlorine levels were noted, indicating that the emesis might be resulted from an imbalance of the electrolytes.

Summary of clinical chemistry findings:

Text Table 9

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Changes in Clinical Chemistry Parameters, as Compared to Pretest Values					
			Total Protein		Albumin		Globulin	
			Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]
Propofol								
1767 F	172.4	-	-31%	-11%	-38%	-18%	-26%	-5%
1766 F	180.0	-	-43%	-10%	-41%	-3%	-45%	-17%
1765 F	226.8	-	-28%	-2%	-30%	+3%	-26%	-7%
1267 M	396.3	-	-29%	0%	-35%	-5%	-23%	+5%
1265 M	819.4	-	NS	NS	NS	NS	NS	NS
1266 M	1091.6	-	-43%	NS	-49%	NS	-38%	NS
GPI 15715								
2765 F	120.9	63.6	-27%	-12%	-24%	-16%	-29%	-8%
2767 F	840.3	442.3	-46%	-27%	-48%	-33%	-45%	-23%
2267 M	907.0	477.4	-31%	-4%	-27%	-2%	-35%	-5%
2766 F	985.1	518.5	-39%	0%	-40%	-10%	-39%	+9%
2265 M	1929.7	1015.6	-39%	-8%	-38%	-14%	-40%	-2%
2266 M	2608.7	1373.0	-41%	NS	-41%	NS	-42%	NS

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Measured during the last hour of infusion.

[c]Measured prior to necropsy.

M = male; F = female; NS = no sample

Text Table 10

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Changes in Clinical Chemistry Parameters, as Compared to Pretest Values					
			Total Calcium		Ionized Calcium		Triglycerides	
			Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]
Propofol								
1767 F	172.4	-	-21%	-9%	NS	NS	+5200%	-4%
1766 F	180.0	-	-19%	-11%	NR	0%	+1100%	-45%
1765 F	226.8	-	-17%	-2%	NS	NS	+7900%	-3%
1267 M	396.3	-	-21%	-8%	NR	+2%	+170%	-67%
1265 M	819.4	-	NS	NS	NS	NS	NS	NS
1266 M	1091.6	-	-23%	NS	-41%	NS	+4500%	NS
GPI 15715								
2765 F	120.9	63.6	-32%	-9%	NS	NS	+1000%	+23%
2767 F	840.3	442.3	-28%	-13%	NS	NS	+700%	+150%
2267 M	907.0	477.4	-29%	-7%	-19%	-6%	+120%	-15%
2766 F	985.1	518.5	-23%	-7%	-4%	+6%	+590%	-48%
2265 M	1929.7	1015.6	-23%	-5%	0%	+6%	+330%	0%
2266 M	2608.7	1373.0	-28%	NS	-8%	NS	+380%	NS

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Measured during the last hour of infusion.

[c]Measured prior to necropsy.

M = male; F = female; NS = no sample

Text Table 11

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Changes in Clinical Chemistry Parameters, as Compared to Pretest Values					
			Aspartate Aminotransferase (AST)		Alanine Aminotransferase (ALT)		Urea Nitrogen (BUN)	
			Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]	Infusion[b]	Necropsy[c]
Propofol								
1266 M	1091.6	-	+2300%	NS	+370%	NS	101%	NS
GPI 15715								
2767 F	840.3	442.3	+420%	+85%	+67%	+140%	+28%	-27%
2266 M	2608.7	1373.0	+460%	NS	+160%	NS	+100%	NS

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9

[b]Measured during the last hour of infusion.

[c]Measured prior to necropsy.

M = male; F = female; NS = no sample

Text Table 12

Animal No.	Total Dose mg/kg	Changes in Clinical Chemistry Parameters, as Compared to Pretest Values			
		Serum Sodium (mEq/L)		Serum Chloride (mEq/L)	
		Pretest	Infusion	Pretest	Infusion
Propofol					
1767 F	172.4	150	138	107	102
1766 F	180.0	148	131	108	95
1266 M	1091.6	151	133	104	97

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9
[b]Measured during the last hour of infusion.
[c]Measured prior to necropsy.
M = male; F = female; NS = no sample

Blood gas analysis: The blood sample for the blood gas analyses were obtained via femoral artery from all animals approximately 8 and 24 hrs of infusion from the 24 hr and 48 hrs infusions group respectively at the last hour of infusion. The animals were not given parental nutrition prior to the blood collection. The blood gas analyses parameters evaluated are listed with the study # 3000-15715-00-06g. There was a decrease in pH values (Sponsor's table #13) apparently related to an accumulation of CO_2 and a compensatory increase in HCO_3. The CO_2 accumulation was attributed to insufficient alveolar ventilation as a result of the depression of the respiratory centers in the brain by the anesthetics. The increase in pH indicates an acidotic condition in the animals.

Summary of blood gas findings:

Text Table 13

Animal No.	Total Dose mg/kg	Total PED[a] mg/kg	Blood Gas Values at Termination of Infusion			
			pH	pCO_2 mm Hg	HCO_3 mmol/L	pO_2 mm Hg
Propofol						
1767 F	172.4	-	7.270	74.4	34.1	43.4
1766 F	180.0	-	7.440	32.8	22.2	48.4
1765 F	226.8	-	7.292	51.9	25.0	61.1
1267 M	396.3	-	7.431	34.3	22.8	559.4
1265 M	819.4	-	NS	NS	NS	NS
1266 M	1091.6	-	NS	NS	NS	NS
GPI 15715						
2765 F	120.9	63.6	7.318	49.8	25.5	89.2
2767 F	840.3	442.3	7.353	44.0	24.4	118.3
2267 M	907.0	477.4	7.357	49.5	27.7	79.6
2766 F	985.1	518.5	7.240	64.6	27.6	95.3
2265 M	1929.7	1015.6	7.339	43.0	23.1	589.9
2266 M	2608.7	1373.0	7.173	106.1	38.9	368.8

[a]Propofol equivalent dose, based on a molecular weight ratio of GPI 15715 to Propofol of 1.9
M = male; F = female; NS = no sample

Urinanalyses: Urine was collected from all animals pretest, during the last hour of infusion, and prior to necropsy. The urinanalyses parameters evaluated are listed with the

study #3000-15715-00-06g. There was a decrease (6-6.5) in the urine pH level after the infusion period in animals treated with propofol as well as GPI 15715 indicating that the kidney is responding to the high hydrogen level in the blood. The urine pH level recovered at necropsy. The specific gravity of urine from all treated animals also decreased which might be attributed to the infusion of large volume of liquid into these animals.

Gross pathology: Complete macroscopic evaluation was performed on all animals. The major findings include thickening of vena cava surrounding the catheter insertion area and enlargement of the iliac lymph nodes. The gross pathological lesions such as enlarged liver, lung containing fluid, red discoloration of inguinal musculature, and periocular edema were noted in all animals which died or euthanized around the infusion period. The presence of pleural effusion, pulmonary fluid, and hepatomegaly was consistent with cardiovascular inefficiency associated with prolonged anesthesia induced by the test articles.

Organ weights: The organ weights reported from this study is listed in the histopathology table below; the major organs were weighed. There were no test article related changes in the organ weights.

Histopathology: Adequate Battery: Yes
Peer review: No
The histopathological examinations were conducted in all tissues from the standard tissue list. The histological findings are restricted mainly to striated muscles such as heart and skeletal muscle indicating that these are the major target organs for toxicity. There were test article related histopathological changes also in the spleen and the skin. Histological lesion in heart consisted of atrial and left ventricular subendocardial myocardial degeneration accompanied by neutrophilic infiltrates corroborating cardiac insufficiency. Some animals surviving to scheduled necropsy had increased neutrophilic infiltrates with myocardiocytes having large nuclei (karyomegaly) with prominent nucleoli. The changes in the skeletal muscles were associated with fibrovascular stroma (perimysium) separating bundles of myofibers accompanied with acute myodegeneration due to neutrophil infiltration. Some animals surviving to scheduled necropsy had distinctive histological changes comprising myofiber loss and conspicuous myofiber regeneration lining the perimysial framework. In some of the propofol treated animals edema in tongue associated with infiltration of neutrophils in the adjacent myofibers. The Sponsor described the finding as ballooning degeneration with marked intracellular edema and overlying glossal epithelium. The histological changes in the spleen consisted of lymphcytosis in the splenic white pulp. This lesion was observed in all treated animals, the lesions were, however, more pronounced among animals that died or were euthanized around the infusion period. The spleen lesions were therefore considered as a treatment related secondary effect. The histological changes in the skin were observed in 1/3 females treated with GPI 15715. This animal had squamous cell hyperplasia, necrosis of the epidermal layer, neutrophilic infiltration with deep mural arteritis, and bacterial contamination. The biological relevance of this isolated finding is not known. Most of the animals treated animals also had tracheal lesions including sub mucosal

inflammation, epithelial loss associated with metaplasia. These changes were attributed to mild local trauma associated with intubation.

Text Table 14: Microscopic Lesions Associated with Intravenous Infusion of Propofol or GPI 15715 in Cynomolgus Monkeys

Animal No.	Total dose (mg/kg)	Total PED[b] (mg/kg)	Sex	Histopathologic findings[a]										
				Heart			Skeletal muscle					Spleen	
				Myodegeneration, with neutrophilic infiltrates	Myocardiocyte karyomegaly	Lymphocytic infiltrates	Hemorrhage	Edema	Degeneration/atrophy/ regeneration	Acute inflammation	Lymphocytic infiltrate	Lymphoid depletion	Follicular lymphocytolysis
Propofol													
1767	172.4	-	F			1					1	2	
1766	180.0	-	F		1							2	
1765	226.8	-	F	1	1							1	
1267	396.3	-	M	1		1			4		2	1	
1265[c]	819.4	-	M	3			4	4	3	3		4	
1266[c]	1091.6	-	M	3			3	3	3			3	3
GPI 15715													
2765	120.9	63.6	F									1	
2767[c]	840.3	442.3	F				1	2	2	1		1	
2267	907.0	477.4	M	1	1	1						2	
2766	985.1	518.5	F	2	2	1			4	2		1	
2265	1929.7	1015.6	M	2	2	1			3			2	
2266[c]	2608.7	1373.0	M	3			4	3				3	2

[a]Histopathology lesion severity grades: 1: minimal; 2: slight; 3: moderate; 4: marked.
[b]Propofol equivalent dose, based on a molecular weight of GPI 15715 to Propofol of 1.9.
[c]Designated animals were unscheduled deaths.

Toxicokinetics: The blood samples were collected during and after infusion of propofol and GPI 15715 as described in the following table from the Sponsor. The toxicokinetic analyses was designed to compare the exposures of propofol after IV administration of propofol itself with the prodrug (GPI 15751) as indicated in the Sponsors table #1. The exposures of propofol (AUC) were comparable **in all animals (Sponsor's table 2). The** Cmax was higher in the propofol treated animals compared to that of the GPI 15715 treated animals. The formate concentrations were similar in all groups. There were no gender differences in the exposures of propofol and formate.

Table 1: Dosing and Plasma Sampling Information for each Monkey on Study

GROUP 1 PROPOFOL									
		Induction			Maintenance			TK Blood Sample Times	
Animal No.	Body Weight (kg)	Dose (mg/kg)	Time Administered	Time TK Sample Collected	Average Dose (mg/kg/hr)	Duration of Infusion (hr)	Total Post Induction Dose Administered (mg/kg)	Post-Onset of Infusion	Post-Termination of Infusion
1765 F	3.0	10	10:32	10:35	30.18	7.48	225.77	4, 7.48 hr (end of infusion)	0.5, 2, 6, 12 hr and 6 days (sacrifice)
1766 F	2.2	10	11:35	11:38	29.83	6.02	179.59	1, 4 hr	0.37 hr and 6 days (sacrifice)
1767 F	2.6	10	10:59	11:00	27.02	6.38	172.38	4, 6.38 (end of infusion)	0.5, 2, 6, 12 hr and 6 days (sacrifice)
1265 M	3.0	10	12:35	12:40	22.29	36.75	819.0	1, 12, 24, 36 hr (animal died during dosing)	-
1266 M	3.0	10	15:28	15:30	25.73	42.33	1089.27	1, 12, 24, 36, 41.9 hr(end of infusion)	-(animal died during recovery)
1267 M	3.2	10	12:47	12:49	16.71	23.92	399.63	1, 4, 12 hr	1, 6, 24 hr and 5 days (sacrifice)

Table 1 (cont'd): Dosing and Plasma Sampling Information for each Monkey on Study

GROUP 2 GPI 15715									
		Induction			Maintenance			TK Blood Sample Times	
Animal No.	Body Weight (kg)	Dose (mg/kg)	Time Administered	Time TK Sample Collected	Average Dose (mg/kg/hr)	Duration of Infusion (hr)	Total Post Induction Dose Administered (mg/kg)	Post-Onset of Infusion	Post-Termination of Infusion
2765 F	2.6	38 [20]	11:36	11:38	30.89 [16.26]	3.9	120.46 [63.4]	4 hr	0.5, 2, 6, 12 hr and 6 days (sacrifice)
2766 F	2.4	38 [20]	11:16	11:19	43.47 [22.88]	22.67	985.42 [518.64]	1, 4, 12, 22.7 hr (end of infusion)	0.5, 2, 8, 24 hr and 5 days (sacrifice)
2767 F	2.6	38 [20]	12:15	12:19	34.84 [18.34]	23.78	828.54 [436.07]	4, 10, 23.9 hr (end of infusion)	0.5, 2, 6, 12 hr and 2 days (sacrifice)
2265 M	3.8	38 [20]	13:46	13:50	36.68 [19.31]	48	1760.84 [926.76]	1, 12, 24, 36, 47.3 hr	1, 6, 24 hr and 5 days (sacrifice)
2266 M	3.2	38 [20]	14:39	Time not recorded	52.11 [27.43]	48	2501.31 [1316.48]	1, 12, 24, 36, 47.6 hr	1 hr (animal sacrificed)
2267 M	3.2	38 [20]	12:07	12:17	37.8 [19.89]	24	907.19 [477.47]	1, 4, 12, 23.8 hr	1, 6, 24 hr and 5 days (sacrifice)

Note: Doses in brackets are the theoretical propofol equivalent doses based on a ratio of 1.9.

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Table 2. Overall Mean (S.D.) Toxicokinetic Parameters for GPI 15715, propofol and formate

GROUP	COMPONENT	C_{max} (µg/mL) (S.D.)	T_{max}* (hours) (range)	AUC_{0-t} (µg.h/mL) (S.D.)	AUC_{0-24}** (µg.h/mL) (S.D.)
1	GPI 15715	ND	ND	ND	ND
PROPOFOL	PROPOFOL	23.2 (16.8)	2.02 (0.02 – 12.00)	230 (122)	200 (49)
(n = 6)	FORMATE	29.6 (6.8)	9.19 (0.05 – 41.90)	2437 (1377)	495 (98)
2	GPI 15715	440 (496)	0.06 (0.03 – 36.00)	3164 (5718)	917 (404)
GPI 15715 (n = 6)	PROPOFOL	18.1 (19.7)	13.90 (0.03 – 36.00)	330 (339)	187 (67)
	FORMATE	30.0 (2.8)	11.00 (0.05 – 147.90)	2680 (1129)	601 (43)

ND = Not Determined - No measurable plasma GPI 15715 concentrations
* median; ** n = 5

Study title: GPI 15715: Two-Week Pilot Intravenous Toxicity Study in Cynomolgus Monkeys

Key study findings

- This is a dose range-finding study. The test article was administered with GPI 15715 as an intravenous bolus induction dose of 38 mg/kg followed by a 42 mg/kg/hr intravenous for 3 hrs/day, 3 times/week for 2 weeks in cynomolgus monkeys (1/sex/group).
- There was a decrease in RBC, hemoglobin, and hematocrit and blood pH and HCO_3 in all animals indicating acidosis.

Study number: 3000-15715-02-02n
Volume # and page #: Module 4-eCTD submission; Page#: 1-121
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 12-02-2002
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715, Lot #312I1002; the test article was supplied as a sterile solution in saline (20 mg/mL)
Vehicle: Saline

Methods

Doses: GPI 15715/Induction 38 mg/kg & maintenance 42 mg/kg/hr/day
Species/strain: *Macaca fascicularis*
Number/sex/group or time point (main study): 1/sex/group
Route and formulation: Intravenous bolus and infusion; GPI 15715 was used as received from supplier.

Satellite groups used for toxicokinetics or recovery: None
Age: Approximately 5 years and 3 months
Weight: Males 3.3 kg; Females 2.6 kg
Sampling times: Toxicokinetic analyses were not done in this study.
Unique study design or methodology (if any): This is a dose range-finding study. The test article was administered as an intravenous bolus induction dose of 38 mg/kg followed by a 42 mg/kg/hr intravenous for 3 hrs/day, 3 times/week for 2 **weeks (refer to the Sponsor's study design table). The animals were mechanically** ventilated during anesthesia; an endotracheal tube was inserted after the induction dose and/or during the start of the maintenance dose. The animals were sacrificed 4-days after the last dose.

Study design table:

	Bolus Dose[a]			Infusion Dose[a]			Number of Animals					
							Total		Clinical Pathology[b] Pretest and Term		Necropsy[c] Day 15	
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	Dose (mg/kg/hr)	Volume (mL/kg/hr)	Conc. (mg/mL)	M	F	M	F	M	F
1 (GPI 15715)	38	1.9	20	42	2.1	20	1	1	1	1	1	1

[a]Doses represent active ingredient.
[b]Hematology, coagulation, clinical chemistry (including free calcium and pH) and blood gas analysis were performed pretest and Study Day 15. Urinalysis was performed pretest and Study Day 14.
[c]Necropsy was performed 4 days following the last dose.

M = Male; F = Female; Conc. = Concentration; mg/kg = milligrams of test article per kilogram of body weight; mL/kg = milliliters of dosing solution per kilogram body weight; mg/mL = milligrams of test article per milliliter dosing solution; mg/kg/hr = milligrams of test article per kilogram of body weight per hour; mL/kg/hr = milliliters of dosing solution per kilogram body weight per hour

The first day of dosing was Day 0 of the study.

Observations times and results

Mortality: The animals were observed in their cages twice daily for mortality and general condition. There was no mortality in this study.

Physical examinations: The animals were examined pretest and at necropsy for evaluating the general condition, skin and fur, eyes, nose, oral cavity, abdomen, and external genitalia as well as evaluation of respiration and observations for any unusual behavior. There were no abnormal findings in the physical examination.

Clinical signs: The clinical signs of anesthesia (parameters indicated in the study #3000-15715-00-05n) were monitored once during the first hour of infusion and approximately 4-6 hrs intervals thereafter. There were light to moderate anesthesia as indicated by the lack of consciousness, voluntary movements, less than normal tone of muscle, occasional

palpebral and pedal reflex, absence of sensitivity to light touch, and sporadic involuntary movement.

Ophthalmological examination: The animals were examined pretest and at the end of the dosing period by indirect ophthalmoscopy to evaluate cornea, anterior chamber, iris, lens, vitreous humor, retina, and optic disc. The eyelids, lacrimal apparatus, and conjunctiva were also examined grossly. There were no ocular abnormalities in the test article treated animals.

Body weight: Body weights were recorded at pre dose and at Days 2, 4, 7, 9, 11, and 15. There were no test article treated changes in body weights or body weight gain.

Food consumption: Food consumption was estimated based on visual examination and scored based on a numerical scale of 0 (no food eaten) to 5 (all food eaten). There were no changes in the food consumption.

Cardiovascular assessment: Cardiovascular data was collected via manual electrocardiography and implanted telemetry devices at pretest and Day 15 in the anaesthetized condition. Blood pressure and heart rates were also assessed at the same time. There were no changes in ECG. A decrease in heart rate and blood pressure were noted in males and females compared to pretest values.

Hematology and coagulation: The blood samples for hematology and coagulation studies were obtained via femoral venipuncture from all animals pretest and at Day 15. The hematology and the coagulation parameters evaluated are listed with the study #3000-15715-00-06g. There was a decrease in erythrocytes and increase in the reticulocytes and platelets in both males and females compared to their pretest values. A increase in the leucocytes and neutrophils were noted in the male compared to its pretest values but not in the female. The significance of the hematology changes is not known.

Summary of hematological findings:

Hematology		
Parameters (% change)	Male	Female
Hemoglobin Hematocrit RBC	10-14↓	8↓
Reticulocytes	29↑	89↑
WBC	47↑	No Change
Neutrophil (absolute count)	226↑	No Change
Platelets	72↑	47↑

Clinical chemistry: The blood samples for clinical chemistry studies were obtained via femoral venipunctutre from all animals pretest and at Day 15. The clinical chemistry parameters evaluated are listed with the study # 3000-15715-00-06g. There were no changes in the clinical chemistry parameters in the test article treated animals.

Blood gas analysis: The blood sample for the blood gas analyses were obtained via femoral vein from all animals pretest and at Day 15. The blood gas analyses parameters evaluated are listed with the study #3000-15715-00-06g. There was a decrease in the pH male (0.08↓) and the females (0.19↓). These changes were accompanied by a slight increase in pCO_2 and decrease in HCO_3 indicating acidosis.

Urinanalyses: Urine was collected from all animals at 16 hrs post last dosing. The urinanalyses parameters evaluated are listed with the study #3000-15715-00-06g. There were no changes in the urinanalyses parameters in the test article treated animals.

Gross pathology: Complete macroscopic evaluation was performed on all animals. Macroscopic findings include black foci in all lobes.

Organ weights: This is a dose range finding study organ weights were not recorded.

Histopathology: The histopathological examinations were not conducted because this is a pilot dose range finding study.

Toxicokinetics: Toxicokinetic analyses were not done in this study.

Study title: GPI 15715 and Formaldehyde: Four-Week Intravenous Toxicity Study in Cynomolgus Monkeys

Key study findings

- Cynomolgus monkeys (3/sex/group) were administered with the induction dose (38 mg/kg) of GPI 15715 or formaldehyde (15.2 mg/kg) by intravenous bolus injection for 30-90 seconds followed by the intravenous infusion of GPI 15715 (42 mg/kg/hr) or formaldehyde (16.8 mg/kg/hr/day) approximately three hour per day/three times/week for one month.
- The toxicokinetic analysis of the GPI 15715 demonstrated similar exposure to the parent compound at Day 0 and Day 28. Similarly the exposure of propofol derived from GPI 15715 at Day 0 was comparable to that at Day 28. The results indicate that there was no accumulation of either the prodrug or propofol. There were no apparent gender differences in the exposure of either propofol or prodrug. The Cmax of the formate level in the body after GPI 15715 administration was comparable to that of the background formate level, however, the Cmax of the formate level in the body after formaldehyde administration was approximately 2-fold higher than that of the background formate level. The total exposures to the

formate as described by AUC appeared to be slightly higher in the formaldehyde and the GPI 15715 treated group compared to that of the control.

- There was a decrease in heart rate and blood pressure in the GPI 15715 treated animals.
- The blood gas analysis showed one male and one female showed acidosis.
- The histological findings were observed in lung, skin, kidney, heart, skeletal muscle, and trachea.
- There was an increased incidence of lymphoid cell aggregates in different tissues such as in the myocardium of heart, lung, femur, and lacrimal gland, eyes, liver and skeletal in different tissues.
- In lungs, in addition to lymphoid cell aggregates, inflammation in the visceral pleura as well as inflammation of the lung vessels was noted. It might be possible that the lymphoid aggregates induced immune response causing inflammation in lungs. The lung is one of the major target organs of toxicity for the test article as noted in the other toxicity studies. The lymphoid aggregates in the tissues mentioned above might be an indication of an inflammatory response produced by the test article.
- Parasitic cysts were observed at different locations in the GI tract which might be an indication of immune suppression associated with the test article administration.
- There was a thickening of the skin surrounding the catheter insertion site in the animals administered with GPI 15715; no such lesions were noted in the propofol treated animals.
- Interestingly, a variety of histological lesions such as hemorrhage, chronic inflammation, hyperkeratosis, and squamous cell hyperplasia were noted in increased incidence in the animals treated with both GPI 15715 and propofol.
- GPI 15715 repeat dose administration might plausibly inducing immune suppression in the monkeys. The chronic inflammation hemorrhages, and hyperplasia of the skin, however, raised some toxicological concerns and might need mechanistic study if chronic application of the test article is to be planned.

Study number: 3000-15715-03-01g
Volume # and page #: Module 4-eCTD submission; Page#: 1-1093 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 01-03-2003
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, Lot # 312I1002; the test article was supplied as a sterile solution in saline (20 mg/mL); Formaldehyde was supplied as crystalline para formaldehyde lot # K08M01 [redacted], purity 95% and dissolved b(4) in aqueous solution by the CRO to obtain an injectable formaldehyde solution.

Vehicle: Saline

Methods

Doses: GPI 15715/Induction 38 mg/kg & maintenance 42 mg/kg/hr/day
Formaldehyde/Induction 15.2 mg/kg & maintenance 16.8 mg/kg/hr/day
Species/strain: *Macaca fascicularis*
Number/sex/group or time point (main study): 3/sex/group
Route and formulation: Intravenous bolus and infusion as stated above, the test article was used as supplied.
Satellite groups used for toxicokinetics or recovery: None
Age: 3.33 years -7.75 years old
Weight: Males 2.9-4.4 kg; Females 2.5-3.7 kg
Sampling times: The blood samples were collected on the first day of dosing and the last day of dosing from the GPI 15715 and the formaldehyde treated animals. At each day, the blood was sampled 5 times (pre dose, after bolus dose administration that is at ~7-8 mins, before the initiation of infusion that is at ~15 mins, 1 hr after the initiation of the infusion, and at the end of the infusion period).
Unique study design or methodology (if any): The test articles either GPI 15715 or formaldehyde was administered as an intravenous bolus induction intravenous infusion for 3 hrs/day, 3 times/week for **4 weeks (refer to Sponsor's study design** table). The animals were mechanically ventilated during anesthesia; an endotracheal tube was inserted after the induction dose and/or during the start of the maintenance dose.

Study design table:

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Group	Bolus Doses[a]			Infusion Doses[a]			Number of Animals									
							Total		Toxicokinetics[b] Days 0 and 25/28[f]		Clinical Pathology[c] Pretest and Days 15 and 28/31[g]		Necropsy[d] Termination (Day 30/32[h])		Microscopic Pathology	
	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	Dose (mg/kg/hr)	Volume (mL/kg/hr)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F
1 (control)[e]	0	1.9	0	0	2.1	0	3	3	3	3	3	3	3	3	3	3
2 (GPI 15715)	38	1.9	20	42	2.1	20	3	3	3	3	3	3	3	3	3	3
3 (Formaldehyde)	15.2	1.9	8	16.8	2.1	8	3	3	3	3	3	3	3	3	3	3

[a]Doses represent active ingredient.

[b]Toxicokinetic samples were collected on the first and last days of dosing (Day 0 and Day 25/28, respectively). Samples were collected from all animals (3 animals/sex/group/time point) predose, immediately after the bolus dose, 7 to 8 minutes the after bolus dose, right before start of infusion (approximately 15 minutes after the bolus dose), 1 hour after the start of infusion, and at the end of infusion (approximately 3 hours after the start of infusion); following the end of infusion, samples were collected at 3 and 24 hours.

[c]Hematology, coagulation, clinical chemistry (including free calcium, pH, creatine kinase isoenzymes, troponin I and troponin T), and blood gas analysis were performed. Urinalysis was also performed pretest and Day 30 for animals in Groups 1 and 3; Day 32 for animals in Group 2.

[d]Necropsy was performed 5 days following the last dose.

[e]Control animals received sterile 0.9% sodium chloride for injection.

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[f]Final toxicokinetic samples were collected on Day 25 for Groups 1 and 3 and on Day 28 for Group 2.

[g]Final clinical pathology samples were collected on Day 28 for Groups 1 and 3 and on Day 31 for Group 2.

[h]Animals in Groups 1 and 3 were sacrificed on Day 30. Animals in Group 2 were sacrificed on Day 32. Animal No. 3901 was terminated on Day 21 due to loss of patency of the vascular access port.

M = Male; F = Female; mg/kg = milligrams of test article per kilogram of body weight. The first day of dosing is Day 0 of the study.

Observations times and results

Mortality: The animals were observed in their cages twice daily for mortality and general condition. There was no mortality in the study. One animal in the formaldehyde group went through unscheduled necropsy because it lost the VAP (vascular access port for the catheter) at Day 2. Therefore formaldehyde was administered via arms and legs and at Day 18 the veins were no longer patent. So the animals were sacrificed at Day 21.

Physical examinations: The animals were examined pretest and at necropsy for evaluating the general condition, skin and fur, eyes, nose, oral cavity, abdomen, and external genitalia as well as evaluation of respiration and observations for any unusual behavior. There were no test article related changes in the physical examination.

Clinical signs: The clinical signs of anesthesia (parameters indicated in the study #3000-15715-00-05n) were monitored in every 30 mins during the infusion period. All animals (except one) showed light to moderate anesthesia as indicated by the lack of consciousness, voluntary movements, less than normal tone of muscle, occasional palpebral and pedal reflex, absence of sensitivity to light touch, and sporadic involuntary movement. One male appeared to be in much lighter plane of anesthesia and at Day 18 it

showed signs of awakening by voluntary movement, so infusion was discontinued in this monkey after an hour on that day. In one female red exudates were noted at the tip of the endotracheal tube after removal of the tube on Days 7 and 16 and a small amount of red fluid was found in the tube on Day 16. On Day 18, in the same animal mucus membrane was purple indicating cyanosis, therefore infusion was discontinued on that day. The time to recovery from anesthesia is shown in the following table from the Sponsor. Mean values in males and females were comparable.

Summary of clinical sign findings:

Animal No.	Righting Recovery Time (min)		Sitting Recovery Time (min)	
	MALE			
	Mean ± SD	Range	Mean ± SD	Range
2401	22 ± 14	8 - 57	27 ± 16	10 - 64
2402	8 ± 3	3 - 11	9 ± 3	3 - 13
2403	23 ± 4	13 - 27	27 ± 6	11 - 35
All Males	17 ± 6	3 - 57	20 ± 8	3 - 64
	FEMALE			
	Mean ± SD	Range	Mean ± SD	Range
2901	13 ± 5	5 - 24	16 ± 6	7 - 25
2902	19 ± 6	12 - 34	21 ± 6	14 - 36
2903	23 ± 7	13 - 27	27 ± 9	16 - 44
All Females	18 ± 5	5 - 34	22 ± 5	7 - 44

Min = minutes; SD = standard deviation

Ophthalmological examination: The animals were examined pretest, Day 17 and 3 days after the final dose by indirect ophthalmoscopy to evaluate cornea, anterior chamber, iris, lens, vitreous humor, retina, and optic disc. The eyelids, lacrimal apparatus, and conjunctiva were also examined grossly. There were no ocular abnormalities in the teat article treated and the formaldehyde treated animals.

Body weight: Body weights were recorded at pre dose and weekly there after. There were no test article treated changes in the body weights.

Food consumption: Food consumption was recorded pretest and was not recorded during anesthesia. There were no test article related changes in the food consumption.

Measurement of % O_2 saturation and % CO_2: Measurements were made at every 30 mins during the infusion period. Oxygen saturation in blood was between 58-100% and CO_2 expiration was within 22-46% at all intervals. The values are within the normal limits in the ventilated animals.

Cardiovascular assessment: Cardiovascular data was collected via manual electrocardiography at Days 17 and 29 via a 7-lead ECG tracing. The blood pressure and heart rates were assessed at Days 15 and 29. Measurements were made and recorded approximately every 2 mins for the first 20-25 mins. Thereafter measurements were recorded at every 30 mins during the infusion period. There were no changes in ECG. The heart rate and the blood pressure decreased during the anesthesia in GPI 15715 treated animals, however, the blood pressure and the heart rate returned to the normal level after the each treatment regimen. The mean values of the heart rate changes in the anaesthetized males and females are reproduced in the following table from the Sponsor.

Summary of heart rate changes:

	% Change from Pretest			% Change from Control		
Group	**Pretest**	**Day 15**	**Day 29/31**	**Pretest**	**Day 15**	**Day 29/31**
MALE						
Control	NA	-11	-8	NA	NA	NA
GPI 15715[a]	NA	-27	-23	2	-16	-14
Formaldehyde	NA	-11	-5	3	3	7
FEMALE						
Control	NA	-12	-25	NA	NA	NA
GPI 15715[a]	NA	-7	15	-17	-12	28
Formaldehyde	NA	-12	24	-21	-21	32

[a]: in unanesthetized monkeys

Hematology and coagulation: The blood sample for hematology and coagulation studies was obtained via femoral venipunctutre from all animals pretest and at post dosing. The hematology and the coagulation parameters evaluated are listed with the study #3000-15715-00-06g. There were no test article related changes in the hematology and coagulation parameters.

Clinical chemistry: The blood samples for clinical chemistry studies were obtained via femoral venipunctutre from all animals pretest and at Days 15, 21, and 28/31. The clinical chemistry parameters evaluated are listed with the study #3000-15715-00-06g. There were no test article related changes in the clinical chemistry parameters at the end of the dosing period; however, at Day 17 of the blood sample an elevation of triglycerides (182%↑) were noted.

Blood gas analysis: The blood sample for the blood gas analyses were obtained via femoral vein from all animals pretest and at Days 17, 21, and 28/31. The animals were not given parental nutrition prior to the blood collection. The blood gas analyses

parameters evaluated are listed with the study #3000-15715-00-06g. In general, there were no test article related changes in the blood gas analyses in the test article treated animals. However, one male which was tolerant to the anesthesia by showing awakening and one female which showed cyanosis at Day 18 were slightly acidotic with an increase of CO_2 and HCO_3.

Urinanalyses: Urine was collected from all animals pretest and prior to necropsy. The urinanalyses parameters evaluated are listed with the study #3000-15715-00-06g. There were no test article related changes in the urinanalyses parameters.

Gross pathology: Complete macroscopic evaluation was performed on all animals. The major findings include thickening of vena cava surrounding the catheter insertion area and enlargement of the iliac lymph nodes. There were no changes in the gross pathology in the test article or the formaldehyde treated animals.

Organ weights: The organ weights reported from this study are listed in the histopathology table below; the major organs were weighed. There was a statistically significant increase in the liver weight (16 %↑) compared to the controls in the test article treated females. The liver weight in the males also showed increase; however, the increase was not statistically significant.

Histopathology: Adequate Battery: Yes
Peer review: No
The histopathological examinations were conducted in all tissues from the standard tissue list. The histological findings were observed in lung, skin, kidney, heart, skeletal muscle, and trachea indicating that these are the major target organ for toxicity. There was an increase in the incidence of lymphoid cell aggregates in different tissues such as in the myocardium of heart, lung, femur, and lacrimal gland, eyes, liver, and skeletal muscle. The nature of the aggregates was not examined. In lungs, in addition to lymphoid cell aggregates, inflammation in the visceral pleura as well as inflammation of the lung vessels was noted. The lung is one of the major target organs of toxicity for the test article as noted in the other toxicity studies. Parasitic cyst was observed at different location in the GI tract which might be an indication of immune suppression associated with the test article administration. There was a thickening of the skin surrounding the catheter insertion site in the animals administered with GPI 15715; similar lesions were noted in the formaldehyde but not saline treated animal in general. The finding suggests the test article related injection site reaction. The biological relevance of such findings is not known. A variety of histological lesions such as hemorrhage, chronic inflammation, hyperkeratosis, hypertricohsis and squamous cell hyperplasia was noted in increased incidence in the animals treated with GPI 15715 and propofol. Interestingly, the hyperkeratosis and hypertricohsis was noted to occur after the administration of immunosuppressive compound such as cyclosporine.

Summary of gross lesions & histopathological findings:

Parameters	Saline	GPI 15715	Formaldehyde

Eye/Lymphoid cell aggregates	0/6	1/6 1F, minimal	1/6 1M, minimal
Lacrimal gland/Lymphoid cell aggregates	0/6	1/6 1F, minimal	0/6
Injection site/Catheter insertion area/Vein: intima/hyperplasia	2/6 1M, slight 1F, minimal	4/6 3M, minimal 1F, slight	0/6
Injection site/Catheter insertion area/Vein: chronic inflammation	0/6	1/6 1F, minimal	0/6
Injection site/Catheter insertion area/Vein: fibrosis	0/6	3/6 1M, slight 2F, slight	0/6
Parasitic Cyst/present Cecum Colon Ileum	0/6 2/6 1M 1F	3/6 1M 2F 3/6 1M 2F 1/6 1M	2/6 1M 1F 1/6 1F
Heart Myocardium /lymphoid cell aggregates	3/6 2M, minimal 1F, minimal	3/6 1M, minimal 1M, slight 1F, minimal	0/6
Femur/Microgranuloma	1/6 1M, minimal	3/6 1M, minimal 2F, minimal	0/6
Kidney/Mineral deposits in medulla	2/6 1M, minimal 1F, minimal	3/6 1M, minimal 2F, minimal	0/6
Liver/Lymphoid cell aggregates	0/6	4/6 2M, minimal 2F, minimal	0/6
Lung/Visceral-pleura/chronic inflammation	0/6	1/6 1M, minimal	0/6
Lung/Vessel-chronic inflammation	0/6	1/6 1M, minimal	0/6
Lung/Fibrosis	0/6	1/6 1M, minimal	0/6
Skeletal muscle/Lymphoid cell aggregates	0	1/6 1F, minimal	0/6
Skin/Epithelium/Squamous cell hyperplasia	1/6 1M, minimal	3/6 1M, minimal 1M., slight 1F, minimal	3/6 1M, slight 1F. slight 1F, minimal
Skin/Epithelium /Hyperkeratosis	1/6 1F, minimal	3/6 2M, minimal 1F, minimal	3/6 1M, minimal 1F, minimal
Skin/Hypertrichosis	1/6	2/6	5/6

	1F, moderate	1M, slight; 1F, moderate	2M, slight 3F, slight
Skin/Chronic inflammation	1/6 1M, minimal	3/6 2M, slight 1F, moderate	3/6 1M, minimal 2F, slight
Skin/Hemorrhage	2/6 1M, moderate 1F, slight	4/6 2M, moderate 1F, slight 1F, moderate	3/6 2M, moderate 1F, moderate

Note: M: male; F: female; severity index: minimal, moderate etc

Toxicokinetics: The blood samples were collected during and after the infusion of propofol and GPI 15715 as described in the following table from the Sponsor. The toxicokinetic analysis of the GPI 15715 demonstrated similar exposure as described by Cmax and AUC at Day 0 and Day 28. Similarly the exposure of propofol derived from GPI 15715 at Day 0 was comparable to that of Day 28. The results indicate that there was no evidence of accumulation of either the prodrug or propofol. There were no apparent gender differences in the exposure of either propofol or prodrug. The toxicokinetic analysis was conducted from all animal in the control animals to obtain the background level of formate in the body. The table below shows that the Cmax of the formate level in the body after GPI 15715 administration was comparable to that of the background formate level; however, the Cmax of the formate level in the body after formaldehyde administration was approximately 2-fold higher than that of the background formate level. The total exposures to the formate as described by AUC appeared to be slightly higher in the formaldehyde and the GPI 15715 treated group compared to those of the control animals.

Summary of toxicokinetics analyses:

Test Compound	Cmax	Tmax	AUC(0-6)	AUC(0-last)
GPI 15751				
Day 0	36±7	1	92±19	92±19
Day 28	46±35	1	92±61	92±61
GPI 15751 derived propofol				
Day 0	5.8±2	3	17±3	18±3
Day 28	5.5±1	2	20±5	24±6
Background formate level				
Day 0	40±13	NR	188±9	851±63
Day 25	31±2	NR	166±10	742±46
GPI 15751 derived formate				
Day 0	43±9	3	214±25	933±12
Day 25	35±6	2	184±27	786±80
Formaldehyde derived formate				
Day 0	68±15	0.5	295±65	841±71
Day 25	70±19	1.52	284±30	855±63

Histopathology inventory (optional)

Study	3000-15715-01-06g		3000-15715-02-02n	
Species	**Dog**	**Dog**	**Monkey**	**Monkey**
Adrenals	*	x	*	x
Aorta		x		x
Bone Marrow smear		x		x
Bone (femur)		x		x
Brain	*	x	*	x
Cecum		x		x
Cervix		x		x
Colon		x		x
Duodenum		x		x
Epididymis		x		x
Esophagus		x		x
Eye		x		x
Fallopian tube		x		x
Gall bladder		x		x
Gross lesions		x		x
Harderian gland		x		x
Heart	*	x	*	x
Ileum		x		x
Injection site		x		x
Jejunum		x		x
Kidneys	*	x	*	x
Lachrymal gland		x		x
Larynx		x		x
Liver		x		x
Lungs		x		x
Lymph nodes, cervical		x		x
Lymph nodes mandibular		x		x
Lymph nodes, mesenteric		x		x
Mammary Gland		x		x
Nasal cavity		x		x
Optic nerves		x		x
Ovaries	*	x	*	x
Pancreas		x		x
Parathyroid		x		x
Peripheral nerve		x		x
Pharynx		x		x
Pituitary	*	x	*	x
Prostate	*	x	*	x
Rectum		x		x
Salivary gland		x		x
Sciatic nerve		x		x
Seminal vesicles		x		x
Skeletal muscle		x		x
Skin		x		x
Spinal cord		x		x
Spleen	*	x		

Sternum		x		
Stomach		x		
Testes	*	x		
Thymus		x		
Thyroid		x		
Tongue		x		
Trachea		x		
Urinary bladder		x		
Uterus	*	x		
Vagina		x		
Zymbal gland		x		

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: *Salmonella-Escherichia Coli* / Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with GPI 15715

Key findings

- GPI 15715 was tested in the Ames Reverse Mutation Assay at concentrations of 79.1, 211, 632, 1897 and 5270 µg/plate. There was no evidence for cytotoxicity or precipitate at any concentration. Under the conditions of the study, GPI 15715 was concluded to be negative in the bacterial reverse mutation assay.

Study number: 3000\ 15715-tox-00-03g

Volume # and page #:	Module 4-eCTD submission; Page#: 1-29
Conducting laboratory and location:	[redacted] b(4)
Date of study initiation:	August 21, 2000
GLP compliance:	Yes
QA reports:	Yes
Drug, lot #, and % purity:	GPI 15715; Lot #21708-21-3; 84.8%

Methods: The assay was conducted in two phases using the plate incorporation method. The first phase was the toxicity-mutation assay used to establish the dose-range for the mutagenicity assay and get a preliminary mutagenicity evaluation. The second phase was the mutagenicity assay. Dosing was adjusted to compensate for test article activity. Tester strain TA98 and TA1537 are reverted from histidine dependence to histidine independence by frame shift mutagens. Tester strain TA1535 is reverted by mutagens that cause base pair substitution. Tester strain TA100 is reverted by mutagens that cause both frame shift and base pair substitution mutations. Specificity in *E.coli* is sensitive to base-pair substitution mutations, rather than frame shift.