CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-244

PHARMACOLOGY REVIEW(S)

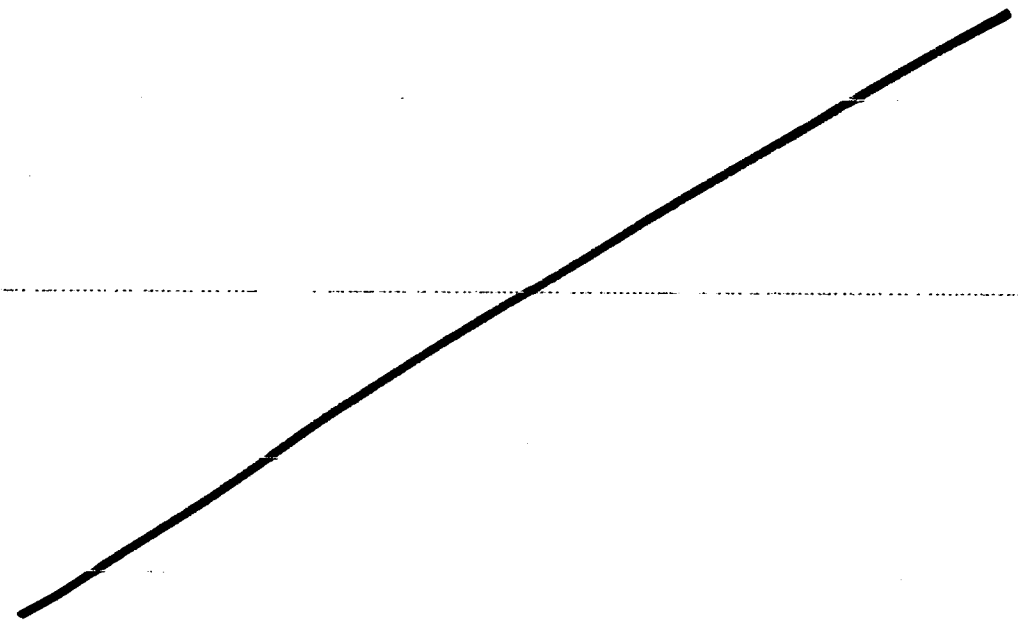
Tertiary Pharmacology/Toxicology Comments on Labeling

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-244
Submission date: September 27, 2007, and subsequent submissions
Drug: fospropofol
Sponsor: MGI Pharma
Indication: monitored anesthesia care sedation in adult patients undergoing diagnostic or therapeutic procedures

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

As noted by the pharmacology/toxicology supervisor, the second cycle submission for this NDA did not include any new pharmacology or toxicology data. As such, the recommendations from the primary reviewer and supervisor provided during the first cycle have not changed. Both the primary reviewer and supervisor determined that the NDA could be approved. I agree.

The sponsor has resubmitted labeling. They have incorporated some changes into the labeling as suggested by the Division. Other recommended changes are shown below. Changes are indicated by red font color. Underlined text has been added and text that is recommended to be deleted is marked with strikethrough.



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/s/

Paul Brown
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PHARMACOLOGIST

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number:	**22-244**
Drug Substance:	**LUSEDRA® (fospropofol disodium) injection**
PDUFA Goal Date:	12-Dec-2008
Sponsor:	**Eisai**
Reviewer name:	R. Daniel Mellon, Ph.D., Pharmacology Toxicology Supervisor
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #:	170
Review completion date:	29-Oct-2008
Recommendation:	**Approval**

The second cycle submission for this NDA did not include any new pharmacology toxicology data. As such, the primary (Dr. Mamata De) and secondary reviewer recommendations provided during the first cycle have not changed. The reader is referred to the labeling recommendations provided by the tertiary pharmacology toxicology reviewer, Dr. Paul Brown.

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/s/

R. Daniel Mellon
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PHARMACOLOGIST

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-244
Submission date: September 27, 2007
Drug: fospropofol
Sponsor: MGI Pharma
Indication: monitored anesthesia care sedation in adult patients undergoing diagnostic or therapeutic procedures

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

Introductory Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for fospropofol to be sufficient to support its use for acute sedation in adults undergoing diagnostic or therapeutic procedures.

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[redacted]. However, the nonclinical information was still considered adequate, in part, because the durations employed in the pivotal nonclinical studies generally exceeded the clinical indication.

Reproductive and developmental toxicity:
The sponsor has proposed a pregnancy category of B while both the reviewer and supervisor recommend a category of C.

The reviewer and supervisor noted some skeletal effects in a **rat intravenous embryofetal study**. I have reviewed the skeletal alterations reported in the study. Some of these alterations occurred in drug treated animals and not control animals. All the alterations occur with low incidence. No clear dose effect is apparent. An increase in wavy ribs may have occurred in the high dose group (litter incidence of 2, fetal incidence of 3 compared to none in control) but significant toxicity occurred in this group and so it is difficult to conclude that any of the skeletal alterations observed in this group are a direct effect of the drug. Bifid vertebrae were more commonly seen in control than drug treated groups. It is my opinion that this study does not show a clear drug effect although some of the alterations did occur in the low dose group in which maternal toxicity was minimal.

The reviewer and supervisor noted some skeletal effects in a **rabbit intravenous embryofetal study**. Significant maternal toxicity including mortality occurred at all doses of fospropofol. However, only occasional occurrences of variations were noted. Although it may appear that some of these occurred more frequently in drug treated groups, there was no dose response, the frequency was low and such findings would not be unexpected in the presence of maternal toxicity. I conclude that the rabbit study does not show any clear drug-related effect on embryofetal development.

According to the CFR, category B should be assigned to products in which animal studies have failed to demonstrate a risk to the fetus. It is my opinion that the submitted embryofetal studies have not demonstrated a clear risk to the fetus in the absence of maternal toxicity. I shared the bone information from these two embryofetal studies with the other pharm/tox associate directors and they agree that there does not appear to be a clear drug-related effect. I believe that a pregnancy category of B could be acceptable based on the submitted data. Although not necessarily a consideration in differentiating pregnancy category B from C, it is worth noting that even if alterations in skeletal ossification or growth were clearly observed in the embryofetal studies, the relevance of this to the very short exposure in humans would be questionable.

The supervisor notes in his review that propofol (the active component of fospropofol) is neurotoxic in young mice and that this supports pregnancy category C for fospropofol.

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[redacted]

A **peri-/post-natal study** was conducted in rats with doses of 0, 5, 10 and 20 mg/kg. The reviewer concluded that the NOAEL was 10 mg/kg because of an increase in the number of dams with any resorptions in mated F_1 females. Note that these animals were not treated with drug during mating or gestation. The number of F_1 dams with any resorptions were 9/22 (40.9%), 5/25 (20.0%), 9/25 (36.0%) and 13/24 (54.2%) in the 0, 5, 10 and 20 mg/kg groups, respectively. Given that the control was 40.9%, it does not appear clear that the finding was elevated in the high dose group. Other measures of resorptions such as mean total, early and late resorptions were not significantly elevated in the F_1 females from the drug treated F_0 groups. The reviewer also noted that performance of F_1 males in a passive avoidance test appeared to indicate a possible effect on memory. However, the parameter used to assess this (latency) had highly variable results such that in some cases the standard deviation was similar or greater than the means. Consequently, none of the results could be considered statistically different. I conclude that this study did not show an effect of drug treatment on peri-/post-natal development in the rat.

A **fertility study** was conducted in rats. The point estimates of mean sperm count and mean sperm density were lower in the high dose group (20 m/kg) than control. However, there was no effect at lower doses and the variability was large so that that the means were not statistically different. Consequently, it is not possible to conclude that the effects were drug related. Upon examination of females it was noted that the total number of nonviable embryos was higher in the drug treated groups compared to control. However, this did not occur in a dose dependent manner and if the unit of comparison is the number of dams with nonviable embryos then essentially no difference is seen between the control and the mid or high dose. The variability in the number of nonviable

embryos per litter is large such that the results were not statistically different. Therefore, it is not possible to conclude that the drug has any effect on embryo viability. I agree with the pharm/tox supervisor and recommend that the labeling state that this rat study did not show an effect on fertility.

Skin toxicity:
A possible signal for local skin effects at the site of injection was noted in some of the nonclinical studies. This was mostly observed in repeat dose studies or studies that employed prolonged infusions. It appears that these findings were not common in shorter infusions or in single dose studies although the single dose studies did not include histopathology assessments of the skin. In some studies these injection site reactions were observed in animals treated with vehicle as well as with the drug. Local skin effects may be relatively straightforward to detect in clinical studies if they occur. If these effects were not observed in the short term clinical studies conducted to support this NDA then the relevance of the nonclinical findings to the proposed indication appears limited. If longer term use were studied in humans in the future then local toxicity should be monitored and appropriate action taken if it is observed. Additional nonclinical studies of the local effects do not appear to be necessary.

Neurotoxicity:
Published studies with propofol have described neuroapoptosis in the brains of juvenile mice. The reviewer and supervisor have recommended that the toxicity of fospropofol be further examined in a juvenile animal model with particular emphasis on neurotoxicity before studies in pediatric patients below the age of 3 are conducted. The current proposed indication is for use in adults. The NDA does not include juvenile animal data. Therefore, it seems reasonable to recommend such a study before developing the drug in children, especially given the possible concern for neurotoxicity.

Conclusions:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA.

I believe a pregnancy category of B may be appropriate based on the submitted studies.

I concur with the recommendation for collecting juvenile animal data before conducting clinical studies in young pediatric patients.

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/s/

Paul Brown
7/16/2008 02:56:34 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-244**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**27-Sept-2007**
PRODUCT:	**TRADENAME (fospropofol disodium) Injection**
INTENDED CLINICAL POPULATION:	**Monitored anesthesia care (MAC) sedation in adult patients undergoing diagnostic or therapeutic procedures**
SPONSOR:	**MGI Pharma**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Mamata De, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Allison Meyer**

Date of review submission to Division File System (DFS): July 1, 2008

Executive Summary

I. Recommendations

A. Recommendation on approvability

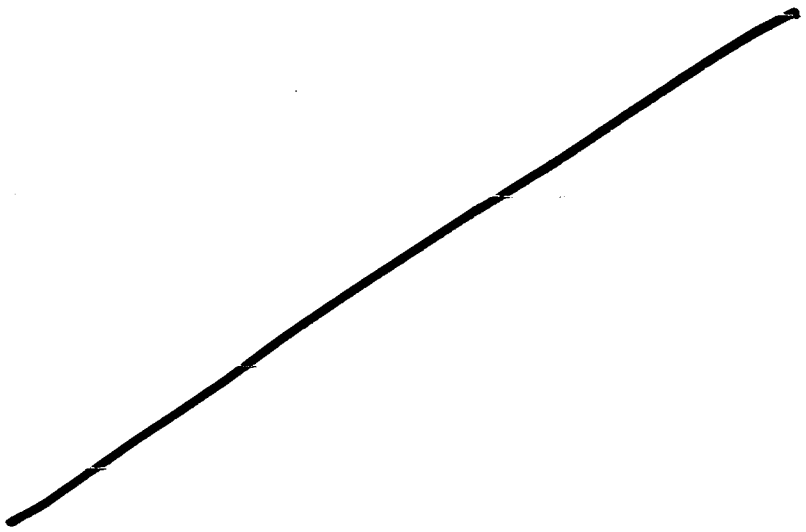
From the nonclinical pharmacology toxicology perspective, NDA 22-204 may be approved, pending agreement on the drug product labeling.

B. Recommendation for nonclinical studies

Prior to studies in pediatric patients 3 years of age and under, developmental neurotoxicology studies should be completed. The Sponsor should specifically address the potential for fospropofol to produce neuronal apoptosis in the developing brain and assess the potential for long-term functional consequences of exposure to fospropofol during brain development, particularly if histopathological findings are noted in the brain.

C. Recommendations on labeling

The following labeling recommendations are undergoing discussion with the Sponsor and may not reflect the final approved drug product labeling.



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II. Summary of nonclinical findings

A. Pharmacologic activity

Fospropofol disodium was developed as a water soluble prodrug of the FDA approved drug propofol. The prodrug does not possess pharmacological activity. Fospropofol is metabolized in the body by alkaline phosphatase to release equimolar equivalents of propofol, formaldehyde, and phosphate (the formaldehyde is further metabolized to formate via aldehyde dehydrogenases). Due to the required metabolic activation of the prodrug, the onset and duration of the sedation produced by fospropofol is delayed and prolonged compared to propofol injections.

The exact mechanism of action of propofol is not entirely clear. There are data in the literature that indicates that propofol potentiates the effects of γ-aminobutryic acid (GABA) through $GABA_A$ receptors. However, there are also data in the literature that suggests that propofol also blocks glycine receptors, neuronal nicotinic receptors, and muscarinic M1 receptors (Trapani et al., 2000).

According to the draft labeling, TRADENAME is administered intravenously as a bolus injection. The standard dosing regimen is an initial dose of 6.5 mg/kg with a supplemental dose of 1.63 mg/kg (25% of the initial dose) as needed. Dosing is limited by lower and upper weight limits (between 60 and 90 kg). The Sponsor stated in the proposed labeling that initial doses should not exceed 16.5 mL and supplemental doses should not exceed 4 mL.

The table below represents the predicted human exposures for the proposed indication of procedural/diagnostic sedation. [redacted] the typical procedure was 10-17 minutes. The submission states that the anticipated human treatment regimen will be a bolus injection followed by 3 to 4 additional bolus doses of up to 1.63 mg/kg each for an anticipated cumulative dose of 13.0 mg/kg (482 mg/m^2); however, there is no maximum duration of exposure proposed by the Sponsor for this indication. Therefore, following discussion with the clinical team, it was estimated that the clinical use of the product for procedural or diagnostic sedation could reasonably be expected to be approximately 30 minutes in some situations. The Sponsor does not have pharmacokinetic data for the various durations of likely clinical use; however, following discussion with the clinical pharmacology and biopharmaceutics reviewers, the clinical Cmax does not appear to change with subsequent bolus infusions and the AUC values appear linear. Therefore, in order to calculate likely exposure margins in order to compare the animal data to the human, the following values were employed.

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Procedure time (min)	Fospropofol (mg/kg)	Cumulative fospropofol (mg/kg)	Cumulative fospropofol (mg/m^2)	$AUC_{(0-\infty)}$ (mcg•hr/mL)	Cmax[1] (mcg/mL)
0	6.5	6.5	240.5	19.00 ± 7.2	78.7 ± 15.4
4	1.63	8.13	300.81	23.75	78.7 ± 15.4
8	1.63	9.76	361.1	28.50	78.7 ± 15.4
12	1.63	11.39	421.4	33.25	78.7 ± 15.4
16	1.63	13.02	481.74	38.00	78.7 ± 15.4
20	1.63	14.65	542.05	42.75	78.7 ± 15.4
24	1.63	16.28	602.36	47.50	78.7 ± 15.4
28	1.63	17.91	662.67	52.25	78.7 ± 15.4
30	0.815	18.725	692.825	54.625	78.7 ± 15.4
32	1.63	19.54	722.98	57.00	78.7 ± 15.4

[1] C_{max} data are not available for 6.5 mg/kg. C_{max} value is following 6 mg/kg dose is 78.7 ± 15.4 and did not appear to change following subsequent doses; therefore for the sake of extrapolation, the 6 mg/kg rather than the 6.5 mg/kg dose is used for Cmax.

The blue line in the above table represents the Sponsor's anticipated use of the product; however, the values area extended out to 32 minutes, to illustrate the exposures that can occur if longer procedural time is required.

B. Brief overview of nonclinical findings

The nonclinical general toxicology program for this propofol prodrug was separated into four general categories of general toxicity studies originally intended to mimic the way sedative agents are used clinically and to fulfill the regulatory requirements as per ICHM3. These general categories are as follows:

1. Single-dose toxicology via bolus injection
2. Single-dose toxicology plus continuous infusion for < 24 hours
3. Repeated-dose toxicology plus continuous infusion for > 24 hours
4. Repeated-dose toxicology via intermittent bolus injections/infusions for 14-30 days

The table below, reproduced from the Sponsor's submission summarized the models employed.

Type of Study	Method of Dosing	Species and Strain	Compound Administered
Single Dose Toxicity: Bolus	i.v. bolus	Mice, CD-1 and Rat, Sprague-Dawley	GPI 15715
	i.v. bolus	Monkey, Cynomolgus	GPI 15715, formaldehyde
Single Dose Toxicity: Continuous Infusion <24 h	i.v. bolus + i.v. infusion	Rat, Sprague-Dawley	GPI 15715, propofol
	i.v. bolus + i.v. infusion	Rat, Sprague-Dawley	GPI 15715, propofol
	i.v. bolus + i.v. infusion	Dog, Beagle	GPI 15715
	i.v. bolus + i.v. infusion	Monkey, Cynomolgus	GPI 15715, propofol
Repeated Dose Toxicity: Continuous Infusion >24 h	i.v. bolus + i.v. infusion	Dog, Beagle	GPI 15715
	i.v. bolus + i.v. infusion	Monkey, Cynomolgus	GPI 15715, propofol
Repeated Dose Toxicity: Intermittent Infusion/Bolus + Infusion	i.v. infusion	Rat, Sprague-Dawley	GPI 15715, propofol
	i.v. bolus + i.v. infusion	Dog, Beagle	GPI 15715, propofol
	i.v. bolus + i.v. infusion	Dog, Beagle	GPI 15715, propofol
	i.v. bolus + i.v. infusion	Monkey, Cynomolgus	GPI 15715
	i.v. bolus + i.v. infusion	Monkey, Cynomolgus	GPI 15715, formaldehyde

As noted in the table above, the Sponsor frequently included a comparator arm of either propofol or formaldehyde to account for the toxicological consequences of sedation for prolonged periods in animals. For studies designed to compare fospropofol to propofol, equivalent molar doses were administered (1.86 mg of fospropofol disodium, when completely metabolized, produces 1 mg of propofol).

Although propofol is FDA approved for both acute and long-term sedation in the ICU, [redacted] the current application only proposes an indication of sedation for [redacted] diagnostic and therapeutic procedures in adult patients.

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The Sponsor conducted single dose toxicology studies via bolus infusion in the mouse, rat, and monkey (listed in the sponsor's table below):

Species and Strain	Method of Administration (Vehicle)	Dosages[a]	Sex and No. per Group	Observed Maximum Nonlethal Dosage[a]	Approximate Lethal Dosage[a]	Noteworthy Findings	Study Number	
Single Dose Toxicity: Bolus Dose Studies								
Mouse, CD-1	IV bolus (0.34% saline)	0, 40, 80, 160, 320 mg/kg	5M, 5F	160 mg/kg	320 mg/kg	≥80: dose-related loss of righting reflex (sedated) 320: 5M and 5F died	3000-15715-00-04G	No histopath
Rat, Sprague-Dawley	IV bolus (0.34% saline)	0, 40, 80, 160, 320 mg/kg	5M, 5F	80 mg/kg	160 mg/kg	≥40: sedated 160: 3M and 5F died 320: 5M and 5F died	3000-15715-00-04G	No histopath
Monkey, Cynomolgus	IV bolus (0.4% saline)	38, 44, 50, 56 mg/kg	1M, 1F	56 mg/kg	ND	≥38: loss of righting reflex; decreased blood pressure <50 mm Hg: trembling noted on one occasion in 1M and 1F	3000-15715-02-01G	Inadequate # animals
Single Dose Toxicity: Continuous Infusion <24 h								
Rat, Sprague-Dawley	IV bolus + IV infusion (0.34% saline)	40 mg/kg + 40 mg/kg/h x 4h	2M, 2F	40 mg/kg + 40 mg/kg/h x 4h	ND	All sedated 1 rat (10016) stopped breathing mid way through infusion but was revived	3000-15715-00-08N	Inadequate # animals Non-GLP
Rat, Sprague-Dawley	IV bolus + IV infusion (0.34% saline)	40-60 mg/kg + 70-140 mg/kg/h x 1.7-5.0 h	2M, 2F	ND	40-60 mg/kg + 70-140 mg/kg/h x 1.7-5.0 h	All rats sedated and died on test with mean duration of infusion 3.8 h; ↓WBC, K^+, Ca^{++}, pH, PaO2; ↑PT, APTT, $PaCO_2$; minimal pulmonary edema	3000-15715-00-02N	Inadequate # animals Non-GLP
Dog, Beagle	IV bolus + IV infusion (0.12% TRIS and 0.25% MTG)	38 mg/kg + 70, 80, 90 mg/kg/h x 4.7-6.0 h	3M, 3M, 2M	38 mg/kg + 90 mg/kg/h x 5.6 h	ND	↓BW, FC, BP; ↑HR; abnormal excreta	WIL 458007	Non-GLP Pilot Study
Monkey, Cynomolgus	IV bolus + IV infusion (0.34% saline)	46 mg/kg + 65-71 mg/kg/h x 6 h	1M, 1F	46 mg/kg + 65-71 mg/kg/h x 6 h	ND	↓BP; EEG pattern to delta range with voltage and burst suppression	3000-15715-01-01N	Inadequate # animals Non-GLP

IV= Intravenous; ND = Not Determined; M = Male; F= Female; TRIS = Tromethamine; MTG = Monthioglycerol; BW = Body Weight; FC = Food Consumption; BP = Blood Pressure; HR = Heart Rate; PT = Prothrombin Time; APTT = Activated Partial Thromboplastin Time; WBC= White blood cell(s); K^+= Potassium, Ca^{++}= Calcium; PaO_2= Partial pressure of oxygen in arterial blood; $PaCO_2$= Partial pressure of carbon dioxide in arterial blood; EEG = Electroencephalogram; s.c. = subcutaneous

a. Dosage units are indicated for each study

None of these studies alone are adequate for use to establish a safety margin for the proposed initial bolus injection of the compound as they did not include histopathological analysis of the tissues or did not include an adequate number of animals to allow for a risk assessment.

To support the proposed indication, the sponsor has referenced the following pivotal single-dose and repeat-dose toxicology studies to establish safety margins (table below reproduced from the Sponsor's submission):

Table 2 Safety Margins for Fospropofol Based on Anticipated Cumulative Human Dosages and Clinical Exposures (6.5-mg/kg Single Dose) vs Maximum Cumulative Dosages and Exposures (AUC_{0-t}) in Rats, Dogs, and Monkeys[a]

Type of Study Species-Schedule [Study Number]	Mean Target Dosage: Bolus + Infusion	Target Dosing Duration: Bolus + Infusion	Calculated Total Dosage Fospropofol Disodium (mg/kg)	Calculated Total Dosage Fospropofol Disodium[b] (mg/m^2)	Safety Margin (A:H[c,d,e])		
					(mg/kg)	(mg/m^2)	(AUC_{0-t})
Single Dose Studies							
Dog [WIL-458007]	38 mg/kg + 90 mg/kg/h	1 d + 6 h	578	11,600	44.5	24.1	17.8
Monkey [3000-15715-01-01N]	45.6 mg/kg + 64.6-71.1 mg/kg/h	1 d + 6 h	441	5,290	33.9	11.0	27.3
Repeated Dose Studies							
Rat – 2 h/d for 14 d [3000-15715-00-07G]	No Bolus 47.5 mg/kg/h	N/A 28 h	1,330	7,980	102	16.6	3.58
Dog – 1h/d for 14 d [3000-15715-00-06G]	38 mg/kg + 64.6 mg/kg/h	14 d + 14 h	1,440	28,700	111	59.7	5.79
Monkey – 3 h/d, 3x/wk for 30 d [3000-15715-03-01G]	38 mg/kg + 42 mg/kg/h	12 d + 36 h	1,970	23,600	152	49.1	4.43

[a]presented as dosage per body weight (mg/kg) or body surface area (mg/m^2). Animal exposure data (AUC_{0-t}) are not included in this table, but are presented in Section 2.6.7.3. Sexes were combined for this table. Numbers rounded to 3 significant figures.
[b]conversion factors from mg/kg to mg/m^2 were 6, 20, and 12 for rat, dog, and monkey, respectively.
[c]A:H=ratio of maximum animal dosage to anticipated human dosage (mg/kg) where H=13.0 mg/kg
[d]A:H=ratio of maximum animal dosage to anticipated human dosage (mg/m^2) where H=481 mg/m^2
[e]A:H=ratio of maximum animal exposure to human exposure (AUC_{0-t}) where H=20.8 µg•h/mL based on an extrapolated 6.5-mg/kg dose from clinical study number 3000-0521.

Specifically, the two single dose studies listed in the table above were not conducted under GLPs; therefore, they can not serve as the pivotal studies to support the proposed indication.

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In the **pivotal rat repeat-dose toxicology study** (3000-15715-00-07G), rats were treated fospropofol dose of 47.5 mg/kg/hr for either 1, 2 or 4 hours (there was no bolus induction dose) once a day for 14 days. The Sponsor does not state if they believe a NOAEL was obtained in the study; however, their exposure comparison on the table above compared the 2 hour per day treatment arms to their predicted human exposure of not more than 16 minutes. This implies that they believe the study results at this exposure level are at least a LOAEL. Dr. De concludes that a NOAEL could not be determined since there were injection site reactions at all doses. According to the pathologist's report, these reactions were considered severe but were noted in all groups, including controls. The reaction appears consistent with a foreign body reaction, which is likely due to the presence of the indwelling cannula for prolonged time. There were other apparent treatment-related dose-dependent findings in all

dose groups, including bone marrow cell hyperplasia and spleen extramedullary hematopoesis. These findings are likely due to the repeated injection volumes and blood draws. Kidney congestion was noted at increased incidence in the 2 and 4 hour treatment group as well as the propofol 4 hour treatment group. Although the pathologist noted that these findings are not uncommon in rats and therefore questioned if they were treatment related, I agree with Dr. De that they can not be entirely dismissed. The 4 hour treatment groups (both fospropofol and propofol groups) demonstrated an increased incidence of acute liver inflammation and there were also deaths in both of these groups. As such, in my opinion, the 1-2 hour treatment group can be considered a LOAEL treatment. The animal exposures at 1 and 2 hour per day treatment groups are 0.6 and 1.2-fold the human exposure based on a mg/m^2/day comparison for a 16 minute clinical procedure, respectively. Given the differences in exposure conditions in the study and the proposed human clinical use, the use of body surface area as the basis for comparison provides an acceptable exposure margin. Therefore, in my opinion, this study is adequate to support the NDA application.

In the **pivotal dog repeat-dose toxicology study** (3000-15715-00-06G), dogs were treated with fospropofol via induction dose of 38 mg/kg and a maintenance infusion of 65-95 mg/kg/hr for either 1, 2 or 4 hours once a day for 14 days. This study was designed to compare the effects of fospropofol to propofol, and tested only a single dosing regiment of fospropofol selected to produce mild sedation. As only one dosing regimen for fospropofol was employed, it is not possible to determine a NOAEL in this study. Dr. De identified target organs of toxicity as the bone marrow, lung, injection site, and trachea. The minimal hyperplasia in the bone marrow was comparable in both incidence and severity in both fospropofol and propofol groups. The increased incidence of histological changes in the lung in the fospropofol and propofol groups were attributed to the effects of sedation, although they were reported with a slightly greater incidence and severity in the fospropofol treated animals compared to the propofol treated animals. Likewise, there was a greater incidence of histological changes at the site of injection of fospropofol animals compared to propofol or controls, suggesting that the local tissue reactions in the dog were greater with fospropofol. The changes at the trachea were consistent with intubation and not likely treatment-related. The study report notes that there was more involuntary movements and breathing against the ventilator in the dogs treated with fospropofol compared to propofol, and attributed this to the longer recovery times in the fospropofol treated animals due to the continued conversion of the prodrug to propofol. This could explain the greater incidence of findings in the fospropofol group injection sites, lungs, and trachea. The overall conclusion of the Sponsor is that there were no adverse effects that were unique to fospropofol. The exposures in this study on a daily basis did exceed the proposed human exposure and the study exceeded the duration of the proposed human use without overt signs of toxicity. Although not designed to define both a NOAEL and frank toxicity, since the target organs of toxicity have been identified in other studies in the dog, this study is adequate to support the NDA.

In the **pivotal monkey repeat-dose toxicology study** (3000-15715-03-01G), cynomolgus monkeys were treated with fospropofol (38 mg/kg IV bolus induction followed by 42 mg/kg/hr IV infusion) or formaldehyde (15.2 mg/kg IV bolus induction followed by 16.8 mg/kg IV infusion) for 3 hours per day, up to 3 times per week for up to 4 weeks. This study was designed to compare the effects of fospropofol to formaldehyde (metabolite of

fospropofol), and tested only a single dosing regiment of fospropofol selected to produce light to moderate anesthesia. As only one dosing regimen for fospropofol was employed, it is not possible to determine a NOAEL in this study. Dr. De notes that there was a greater incidence of skin findings in this study for both fospropofol and formaldehyde treated animals compared to controls. These changes were described as hyperkeratosis and squamous cell hyperplasia of the skin epithelium, hypertrichosis, chronic inflammation, and hemorrhage. In general, these changes occurred in both the fospropofol and formaldehyde treatment groups, suggesting that they may be due to the formaldehyde and not a result of sedation or the active propofol formed from fospropofol. This toxicity was not discussed by the Sponsor and the study report does not include a separate pathologist's report; therefore, it is not clear how the reviewing pathologist viewed the findings. Nonetheless, the changes do not appear to have occurred in the clinical setting and therefore may only be evident following repeated administration of the drug. As such, I agree with Dr. De that should the Sponsor pursue an indication where the drug were to be used for sedation in the ICU, further characterization of these findings is warranted.

The overall conclusion of the Sponsor is that there were no toxicologically meaningful findings following administration of fospropofol in this study. The exposures in this study on a daily basis did exceed the proposed human exposure for both a 16 and 32 minute procedure, and the toxicology study exceeded the duration of the proposed human use without overt signs of toxicity. Although not designed to define both a NOAEL and frank toxicity, since the target organs of toxicity have been identified in other studies in the monkey, this study is adequate to support the NDA.

The Sponsor conducted a standard battery of **genetic toxicology studies** which are adequate to support the proposed NDA. The results of the studies suggested that the drug product, under conditions of metabolic activation, was genotoxic in the in vitro mouse lymphoma assay. The Sponsor conducted mechanistic studies; however, to show that the positive finding is negated by inclusion of formaldehyde dehydrogenase. These findings support the hypothesis that the positive finding in vitro is likely an artifact of the build-up of formaldehyde in the culture conditions. As formaldehyde is rapidly metabolized in the body and the in vivo micronucleus assay was negative, the in vitro finding does not raise clinical safety concerns regarding the mutagenic potential of the drug product.

The Sponsor conducted **reproduction and developmental toxicology studies** according to the standard ICH battery. I agree with Dr. De that the study designs likely overestimate the potential toxicity relative to the proposed clinical indication; however, they are designed to cover the entire organogenesis period in order to identify a potential hazard. The only other alternative to this general approach would be to test the drug after single administration on each day of the organogenesis period, which is not feasible.

Segment I (fertility and early embryonic development) studies were completed in the rat model. The potential effects on **male and female fertility** were examined separately in the rat. The Sponsor concluded that there were no effects on fertility in either the males or the females under the conditions of the study. Male rats were treated with 5, 10, or 20 mg/kg fospropofol for 4 weeks prior to mating. Although there was a 15% decrease in mean sperm

count and an 18% decrease in mean sperm density in the high dose males, these changes were not statistically significant and given the variability in the values there is no clear evidence of a treatment-related effect. I concur with the Sponsor's conclusion that at a dose of 20 mg/kg (120 mg/m^2), there were no treatment-related effects on male fertility. This dose is 0.3-fold the total human dose for a procedure of 16 minutes based on a mg/m^2 basis.

Although there were increased preimplantation losses in all treatment groups (5, 10 and 20 mg/kg), the changes were neither statistically significant nor dose dependent. At a dose of 20 mg/kg (120 mg/m^2), there were no clear treatment-related effects on female fertility. This dose is 0.3-fold the total human dose for a procedure of 16 minutes based on a mg/m^2 basis. Both the male and the female fertility studies produced signs of toxicity (decreased body weight gain) in the animals; therefore, the studies are considered valid assessments even if the exposure at the high dose does not completely cover the anticipated human exposure on a mg/m^2 basis. Of note the Cmax values obtained in the males treated with 20 mg/kg (137.7 mcg/mL) did exceed the mean Cmax values clinically (~80 mcg/mL) and the duration of treatment was 2-4 weeks compared to the anticipated 16-30 minute procedure.

Segment II (teratogenicity) studies were completed in both the rat and the rabbit model. Rats were treated with 0, 5, 20, or 45 mg/kg/day fospropofol from GDs 7 through 17. Clear maternal toxicity was evident at doses ≥20 mg/kg. The Sponsor did not identify any adverse events in this study and considers the NOAEL for embryofetal development to be 45 mg/kg/day. There was also an apparent increase in the incidence of pups with incomplete ossification of ribs or sternum. There were no changes noted in the control group of this study and historical control data were not provided. Incomplete ossification is suggestive of a developmental delay and may or may not be secondary to maternal toxicity; however, in the absence of evidence that such changes are not relevant to humans, they must still be considered adverse [redacted] b(4)

Rabbits were treated with 0, 14, 28, 56, or 70 mg/kg/day fospropofol from GDs 6 through 18. Maternal toxicity was noted at all doses, as evidenced by increased mortality. The Sponsor did not identify any adverse events in this study and considers the NOAEL for embryofetal development to be 70 mg/kg/day. Similar to the results of the rat study, there was a suggestion of potential delayed ossification in the rabbit pups from the 28 mg/kg/day treatment groups and above. There was also an apparent dose-related increase in the incidence of displace midline nasal suture in all treatment groups. The dose of 14 mg/kg/day in the rabbit has a human equivalent dose of 168 mg/m^2, or approximately 3-times the human total dose for a 32 minute procedure (57 mg/m^2). Given the evidence of maternal toxicity at all doses, it is possible that these findings may be secondary to maternal toxicity; however, in the absence of evidence that such changes are not relevant to humans, they must still be considered adverse [redacted] b(4)

A **segment III (perinatal and postnatal developmental) study** was completed in the rat model. Pregnant rats were treated with 0, 5, 10 or 20 mg/kg/day fospropofol once daily from gestation day 7 through lactation day 20 (post natal day 20). Pups were allowed to be born

and were therefore exposed to drug in utero and possibly indirectly via breast milk. Developmental parameters evaluated included growth, development, learning and memory, and reproductive performance. According to the Sponsor's interpretation of the study, the NOAEL for maternal toxicity was 5 mg/kg/day. The Sponsor also concludes that the NOAEL for F_1 pup developmental parameters was > 20 mg/kg/day. Dr. De's interpretation of the study differs from that of the Sponsor. Dr. De concludes that the NOAEL for perinatal and postnatal development is 10 mg/kg. This conclusion appears to be based on the finding of increased resorptions in the dams at the high dose compared to controls. However, it is not clear when these resorptions occurred; therefore it is not known if they occurred before drug treatment was initiates or if they occurred after drug treatment was initiated. This is more appropriately determined from the Segment I and Segment II studies. Dr. De concludes that there was an increase in F_1 pup mortality; however, this conclusion is not supported by the study report.

In addition, Dr. De concludes that there was a dose-related decrease in short and long term memory in this study
Based on her review, this conclusion appears to be based on the results of the passive avoidance test. Upon review of the study results from the assay, the mean latency changes are slight and given the standard deviations, it is not possible to draw a definitive conclusion regarding a treatment-related effect.

b(4)

Although there are no data on the potential adverse effects of fospropofol on neuronal development, there are several published reports that have examined the effects of propofol on neuronal development that are relevant to fospropofol.

There are several published in vitro studies that suggest that propofol has the potential to result in neurotoxicity (Honegger & Matthieu, 1996; Zhu et al., 1997; Spahr-Schopfer et al., 2000; Al-Jahdari et al., 2006). There are at least two studies in the published literature that have examined the potential neurotoxicity of propofol in vivo.

Fredriksson et al. reported that administration of 0, 10, or 60 mg/kg of propofol to 10-day old mice via subcutaneous injection and examined the brain for evidence of neurodegeneration 24 hours later. Separate mice were tested for long-term behavioral changes (spontaneous behavior, radial arm maze, and elevated plus maze) at 55-70 days of age. Treatment with the 60 mg/kg dose of propofol increased Fluoro-Jade staining in the olfactory bulb and stria terminalis, indicating an increase in neuroapoptosis in these structures. The lower dose of propofol did not reveal histopathological evidence of neurodegeneration. Post-natal day 10 Propofol treatments did not result in any change in spontaneous behavioral variables (locomotion, rearing and total activity) in 55-day old mice. Likewise, post-natal day 10 propofol treatments did not alter improvement in radial arm maze acquisition performance. In contrast, the anxiolytic effect of diazepam was reduced in mice neonatally exposed to both doses of propofol, suggesting that even in the absence of histopathological evidence of neurodegeneration, mice exposed to propofol during the brain growth spurt showed long-term differences in GABAergic function (Fredriksson et al., 2007). Although pharmacokinetic data are not available in the mouse from this published study and the route of administration is

different than the clinical route, the doses tested in the mouse were 30 and 180 mg/m^2, which are below the proposed clinical dose of propofol from fospropofol for either a 16 or 32 minute procedure (~267.8 or 401.7 mg/m^2, respectively).

Cattano et al. reported that intraperitoneal administration of ≥50 mg/kg propofol to 5-7 day old mouse (but not 25 mg/kg) increases the incidence of neuroapoptotic cells in the brain. The authors demonstrated that a dose of 150 mg/kg, IP resulted in 50% of the mice to lose their righting reflex and a dose of 200 mg/kg, IP was necessary to induce a surgical plane of anesthesia in the infant mouse (50% unresponsive to painful stimuli). Lower doses were reported to produce sedation in a dose-dependent manner. When brain slices were examined 6 hours after propofol treatment, there was a significant increase in the number of activated caspase-3 stained neurons in the cortex and caudate nuclei at doses of 50 mg/kg and greater in a dose dependent manner (Cattano et al., 2008). Although pharmacokinetic data are also not available in the mouse from this published study and the route of administration is different than the clinical route, the minimally effective dose tested in the mouse (50 mg/kg or 150 mg/m^2) is below the proposed clinical dose of propofol from fospropofol for either a 16 or 32 minute procedure (~267.8 or 401.7 mg/m^2, respectively).

Although the clinical significance of these findings are not clear (Mellon et al., 2007), these data suggest that use of propofol or fospropofol during the critical period of brain development (third trimester to 2-3 years of age) should be done only if the potential benefit justifies the potential risk to the fetus. These data support the conclusion that fospropofol should be a Pregnancy Category C drug and that further definitive studies on the potential for neonatal apoptosis should be completed before studies in pediatric patients below the age of 3 are conducted.

C. Nonclinical safety issues relevant to clinical use

The single-most difficult challenge with this drug product application is that the proposed indication of procedural or diagnostic sedation requires only a short duration of exposure to the drug product. The nonclinical single-dose toxicology studies conducted to date are not adequate to allow clear extrapolation of adverse events from the nonclinical program to support such an indication. They do not mimic the proposed clinical dosing regimen. Rather, the program was designed to characterize the potential toxicity of more prolonged durations of exposure. As such, although the toxicities noted in the animal studies clearly define the potential toxicity of this compound, they are not readily extrapolatable to the proposed clinical indication. Ideally, a portion of the pivotal nonclinical toxicology studies would mimic the proposed clinical use of the drug product, and include observations at both an acute and delayed time point. Although not ideal, the repeat-dose toxicology studies conducted, together with the non-pivotal studies that help define the potential extent of toxicity provide an adequate characterization of toxicity, particularly in light of an adequate safety profile from the clinical studies conducted to date. Although the repeat dose toxicology studies do define a NOAEL or LOAEL, the safety margins in the monkey and the dog are adequate based on a mg/m^2 comparison.

Due to the difficulty in extrapolating the animal exposures to the proposed human exposures, comparisons based on body surface area, although not ideal, appear to be the best option. This approach was also employed for the FDA approved propofol products. The Sponsor appears to be proposing exposure margins based on the anticipated 16 minute procedure. However, if a 30-32 minute procedure is likely to occur, the exposure margins will be smaller.

	Initial Bolus Dose	**Supplemental Dose**	**Cumulative Dose (Safety Margin per day)**	
			16 min procedure	**32 min procedure**
Adult Human	6.5 mg/kg 240.5 mg/m^2 Cmax ~80 mcg/mL $AUC_{(0-\infty)}$ ~19 mcg.h/mL	1.6 mg/kg every 4 minutes 59.2 mg/m^2	482 mg/m^2 Cmax ~80 mcg/mL $AUC_{(0-\infty)}$ ~38 mcg.h/mL	722 mg/m^2 Cmax ~80 mcg/mL $AUC_{(0-\infty)}$ ~57 mcg.h/mL
Rat (Pivotal 14-day Toxicity) Study # 3000-15715-00-07G		47.5 mg/kg/h (1 hour)	47.5 mg/kg/d 285 mg/m^2/d (0.6-fold on a mg/m^2 basis) Cmax ~33-41 mcg/mL $AUC_{(0-\infty)}$ ~65-109 mcg.h/mL	(0.4-fold on a mg/m^2 basis)
		47.5 mg/kg/hr (2 hours)	95 mg/kg/d 570 mg/m^2/d (1.2-fold on a mg/m^2 basis) Cmax ~22-29 mcg/mL $AUC_{(0-\infty)}$ ~24-25 mcg.h/mL	(0.8-fold on a mg/m^2 basis)
Dog (Pivotal 14-day Toxicity Study) Study # 3000-15715-00-06G	38 mg/kg 760 mg/m^2 (1.6-fold the 16 min procedure)	64.6 to 94.6 mg/kg/h 1292-1892 mg/m^2/h	102.6 mg/kg/d 2052.0 mg/m^2/d (4.25-fold on a mg/m^2 basis) Cmax ~221-292 mcg/mL $AUC_{(0-\infty)}$ ~85-138 mcg.h/mL	(2.8-fold on a mg/m^2 basis)
Monkey (Pivotal 30-day Toxicity Study) Study # 3000-15715-03-01G)	38 mg/kg 456 mg/m^2/day (0.9-fold the 16 min procedure) Cmax ~ 46 mcg/mL AUC ~ 92 mcg.h/mL	38-79 mg/kg/h	173 mg/kg/d 2076 mg/m^2/d (4.3-fold on a mg/m^2 basis)	(2.9-fold on a mg/m^2 basis)
Rat Segment I (fertility-TK from males only) Study 1707-007	20 mg/kg 120 mg/m^2 (0.3-fold the 16 min procedure) Cmax ~ 137.7 mcg/mL $AUC_{(0-\infty)}$ ~ 14.8 mcg.h/mL		(0.3-fold on a mg/m^2 basis)	(0.17-fold on a mg/m^2 basis)
Rat Segment II Study # 3000-15715-01-05G	5 mg/kg 30 mg/m^2 Cmax ~ 1.6-5.3 mcg/mL $AUC_{(0-\infty)}$ ~ 29-99 mcg.h/mL		(0.06-fold on a mg/m^2 basis)	(0.04-fold on a mg/m^2 basis)

	Initial Bolus Dose	Supplemental Dose	Cumulative Dose (Safety Margin per day)	
			16 min procedure	32 min procedure
Rabbit Segment II Study # 3000-15715-01-05G	14 mg/kg 168 mg/m^2 Cmax ~ 2.5-4.6 mcg/mL $AUC_{(0-\infty)}$ ~ 55-76 mcg.h/mL		(0.3-fold on a mg/m^2 basis)	(0.2-fold on a mg/m^2 basis)
	28 mg/kg 336 mg/m^2 Cmax ~ 14.6-17.5 mcg/mL $AUC_{(0-\infty)}$ ~ 242-307 mcg.h/mL		(0.7-fold on a mg/m^2 basis)	(0.5-fold on a mg/m^2 basis)
Rat Segment III Study # 1707-006	20 mg/kg 120 mg/m^2		(0.1-fold on a mg/m^2 basis)	(0.08-fold on a mg/m^2 basis)

In addition, the Sponsor has designed their nonclinical program to include a positive control of propofol, an FDA-approved drug product. Overall, Dr. De concludes that with the exception of skin changes, the toxicity profile of fospropofol is comparable to that of propofol. I agree with Dr. De that the skin changes noted in the repeat-dose toxicology studies may not have clinical significance for the proposed indication of procedural/diagnostic sedation; however, these changes should be further characterized should the Sponsor seek a more prolonged clinical use indication.

The embryo-fetal development studies in the rat and the rabbit both suggest that fospropofol has an effect on bone ossification. As noted by Dr. De, it is not known if these findings would occur following exposure via the intended clinical indication of procedural and/or diagnostic sedation. Although the irregular ossification noted may be due to maternal toxicity and may only indicate a developmental delay, it is not clear that these changes may not have an effect on function. Further, the daily exposures obtained in the nonclinical embryo-fetal development studies are below the proposed clinical exposure when compared on a body surface area basis. Collectively, the studies do not support the Sponsor's proposed Pregnancy Category B.

Given the accumulating nonclinical data regarding the potential effects of anesthetic agents on the development of the central nervous system, the Sponsor should conduct developmental neurotoxicology studies prior to clinical studies of fospropofol in pediatric patients 3 years of age and below. The published information with propofol also supports a Pregnancy Category C for this drug.

Dr. De recommends that juvenile animal studies be completed to support a pediatric indication. I concur that additional data should be obtained for pediatric patients 3 years of age and under, with particular emphasis being placed on the potential for developmental neurotoxicity.

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/s/

R. Daniel Mellon
7/1/2008 07:41:41 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-244**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09/26/07**
PRODUCT:	**Aquavan (fospropofol disodium injection)**
INTENDED CLINICAL POPULATION:	**Adult patients requiring sedation for diagnostic, therapeutic, [redacted]** b(4)
SPONSOR:	**MGI Pharmaceuticals Inc.**
DOCUMENTS REVIEWED:	**eCTD Module 2 and 4**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Mamata De, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Allison Meyer**

Date of review submission to Division File System: June 26, 2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY **3**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW **19**

2.6.1 INTRODUCTION AND DRUG HISTORY **19**

2.6.2 PHARMACOLOGY **25**

2.6.2.1 Brief summary: 25
2.6.2.2 Primary pharmacodynamics 28
2.6.2.3 Secondary pharmacodynamics: 32
2.6.2.4 Safety pharmacology 35
2.6.2.5 Pharmacodynamics drug interactions: 41

2.6.3 PHARMACOLOGY TABULATED SUMMARY **41**

2.6.4 PHARMACOKINETICS **41**

2.6.4.1 Brief summary 41
2.6.4.2 Methods of Analysis 43
2.6.4.3 Absorption 43
2.6.4.4 Distribution 52
2.6.4.5 Metabolism 58
2.6.4.6 Excretion 63
2.6.4.7 Pharmacokinetic drug interactions 65
2.6.4.8 Other Pharmacokinetic Studies 65
2.6.4.9 Discussion and Conclusions 65
2.6.4.10 Tables and figures to include comparative TK summary 67

2.6.5 PHARMACOKINETICS TABULATED SUMMARY **67**

2.6.6 TOXICOLOGY **67**

2.6.6.1 Overall toxicology summary 67
2.6.6.2 Single-dose toxicity 78
2.6.6.3 Repeat-dose toxicity 87
2.6.6.4 Genetic toxicology 141
2.6.6.5 Carcinogenicity 154
2.6.6.5 Reproductive and developmental toxicology 154
2.6.6.6 Local tolerance 176
2.6.6.8 Special toxicology studies 184
2.6.6.9 Discussion and Conclusions 186
2.6.6.10 Tables and Figures 191

2.6.7 TOXICOLOGY TABULATED SUMMARY **191**

OVERALL CONCLUSIONS AND RECOMMENDATIONS **191**

APPENDIX/ATTACHMENTS **192**

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: From the nonclinical pharmacology and toxicology perspective, NDA 22-244 may be approved.

B. Recommendation for nonclinical studies: None; however, the fospropofol sodium is recommended to be examined in the juvenile studies, prior to its approval in the pediatric population.

C. Recommendations on labeling: The labeling recommendations are noted below. Final labeling can be found in the action letter.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:

Following is the current labeling proposal from the Sponsor:

[redacted]

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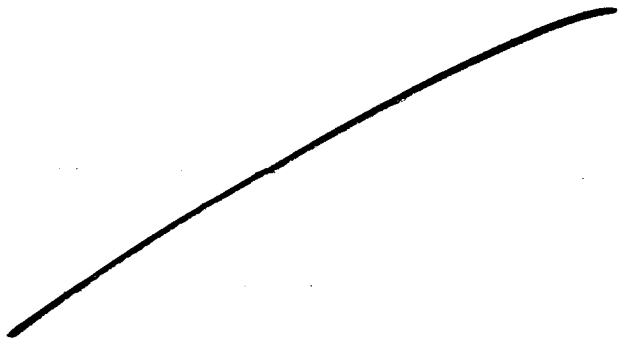
[redacted]

[redacted]



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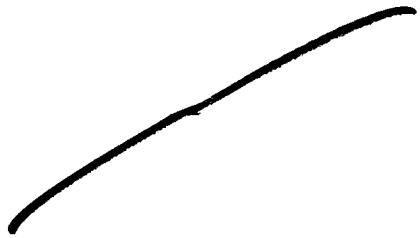


Fertility: In fertility studies in which male rats were intravenously administered fospropofol (5 to 20 mg/kg) prior to and during mating with untreated females, a number of adverse reproductive and developmental effects were observed. These included decreased sperm counts, sperm density, and an increased perimplantation loss. The no-effect dose for male reproductive toxicity in these studies (10 mg/kg, HED=9.6 mg/m^2) was associated with a plasma fospropofol exposure (AUC) approximately 0.05 x human exposure [redacted] In a fertility study in which female rats were given fospropofol (5 to 20 mg/kg) intravenously prior to and during mating and early gestation an increase in the non viable embryos (2-3 fold) was noted. Therefore, a no-effect dose for female reproductive toxicity in rats was established as <5 mg/kg/day (HED=4 mg/m^2).

b(4)

The fertility and the perinatal studies demonstrated an increase in embryo resorptions and nonviable embryos in the test article treated dams compared to the experimental and

historical controls indicating that the above mentioned findings are test article related. NOAEL < 5 mg/kg; HED = 0.8 mg/kg
The embryo-fetal development study in rats and rabbits demonstrated increased skeletal variations such as wavy ribs and incomplete ossifications in all of the test article treated animals compare to the experimental and historical controls suggesting a test article related effect. NOAEL = 20 mg/kg; HED = 3.2 mg/kg.

The embryo-fetal development study in rabbits demonstrated a dose related increase in the mid line suture formation in the nasal area in the fetuses, and an increase in the angulated hyoids in the fetuses from the test article treated dams. Because the increase in the variations was higher than the experimental and historical controls, the findings were considered test article related. NOAEL = 14 mg/kg; HED = 4.5 mg/kg

There were 3 fetuses, one in the 28 and two in the 56 mg/kg/day dose group in the rabbit embryo fetal development study with gross external malformations associated with the soft tissue and skeletal tissue alterations:

- One fetus 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.
- One fetus in the 56 mg/kg/day dose group had two meningoceles, 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, had meningocele in the head. Skeletal evaluation revealed incompletely ossified parietal and frontal bones in the skull

The perinatal development study in rats demonstrated a dose related decrease in the short and long term memory in the F1 males, the biological significance of this finding is not known. NOEL<5mg/kg

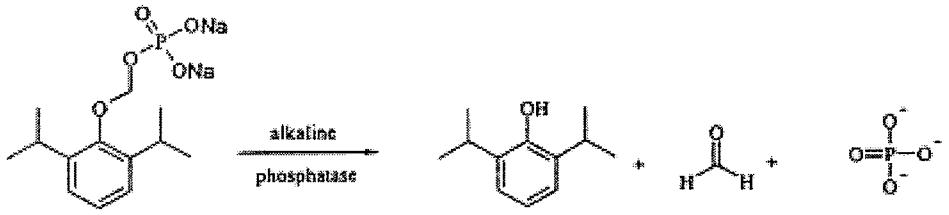
There are no adequate and well-controlled studies in pregnant women. Aquavan should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

II. Summary of non clinical findings

A. Brief overview of non clinical findings:

Fospropofol disodium is the O-phosphonomethyl prodrug form of propofol. The chemical structure of propofol was modified with the addition of chemical moiety to make the molecule water soluble. As shown in the figure below upon enzymatic digestion the phosphonomethyl group is cleaved to yield propofol the active moiety and the other metabolites, formaldehyde and phosphate. The rationale for developing the aqueous formulation is that the peak concentration of propofol from prodrug would be much lower than its lipid formulation. This will induce gradual rise of propofol blood

level which might be associated with less cardio respiratory changes than propofol emulsion.



Fospropofol disodium is proposed for short term sedation with a single bolus and continuous intravenous administration of the test article. b(4)

In order to obtain the marketing license for fospropofol disodium, the Sponsor completed the appropriate nonclinical studies including repeat and single dose general toxicity studies in rat, dog, and monkeys, genotoxicity studies, and reproductive toxicity studies.

The single dose studies were conducted in the rat, dog, and monkey. There were no significant toxicity findings from the single dose toxicity studies. The single dose toxicity studies were most often conducted either to find an appropriate dose or to reach the maximum tolerated bolus dose for intravenous administration of the fospropofol or as a pilot experiment for dose range finding for the intravenous bolus and continuous infusion studies. As a result, the single dose toxicity studies in general did not include adequate number, inclusion of males and females and sometimes did not mimic the clinical protocol.

Therefore the toxicity evaluation of single administration of fospropofol disodium was based on single as well as the repeat dose toxicity studies. In the repeat dose studies fospropofol, unlike the proposed clinical settings, was administered multiple times which resulted in exaggerated toxicity. The repeat dose toxicity, however, noted frank toxicity related to the test article administration.

The clinical pathology findings from all of the toxicity studies consisted of a decrease in the following erythrocytic parameters: RBC, hemoglobin and hematocrit. There was evidence of respiratory acidosis associated with fospropofol disodium administration. An increase in the bicarbonate level and a decrease in blood pH were also noted. All these changes in the blood gas analyses indicate that there might be depression in the respiratory centers resulting in insufficient alveolar ventilation and CO_2 accumulation. The cardiovascular assessment with fospropofol disodium indicate a decrease in heart rate (HR) and mean arterial pressure (MAP) in the monkeys and rats and an increase in HR and MAP in dogs. Similar changes were also noted in the propofol treated animals indicating that the changes are related to the anesthetic property of the test article.

Repeat dose toxicity studies were conducted in Sprague Dawley rats, beagle dogs, and cynomolgus monkeys. Note that the study designs were different in different species. In monkeys and dogs, unlike rats the dosing regimen of bolus and continuous intravenous administration of the test article mimics the clinical dosing regimen.

In these repeat dose toxicity studies, the major toxicity findings were mostly similar in all of the nonclinical species studied. The histopathological lesions consisted of inflammation in lungs, lymphocyte aggregates in liver and heart, bone marrow cell hyperplasia were observed in all species. The histological lesion of similar nature was also observed in animals with propofol treatment in general. The incidence of the lesions or the severity of the lesions varied with the exposure of the test article.

Summary of repeat dose toxicity findings:

Study*/Dosing regimen; Number of animals (n)/ Dose (mg/kg) Exposure: Fospropofol /AUC (µg.h/mL) Exposure: Propofol /AUC (µg.h/mL)	**Major Findings**
Monkey: 38 mg/kg bolus followed by 42 mg/kg infusion/hr/ for 3hr/day, 3x/week for 4 weeks; n= 3/sex/group TDI= 165 mg/kg/day Fospropofol AUC: 91 Propofol AUC: 24 HED= 53 mg/kg/day	**Lymphoid cell aggregates in lungs associated w/inflammation), heart, liver, and skeletal muscle in monkey; Chronic inflammation in lungs in rat and dog; Acute inflammation in liver in rat; Cardiomyopathy@ all doses in rat, severity index highest at mid dose; Parasitic cyst in GI tract in monkey; Congestion in kidney in rat, dog, and monkey Skin disorders such as squamous cell hyperplasia, hypertrichosis, hemorrhage; in monkey Skin thickening in the injection site in dog; Chronic active inflammation in the injection site in rat; Bone marrow cell hyperplasia in rat and dog Spleen extramedullary hematopoesis in rat;**
Dog: 38 mg/kg bolus followed by 95 mg/kg infusion/hr/day for 14-days; n= 3/sex/group TDI= 133 mg/kg/day Fospropofol AUC: 96 Propofol AUC: 24 HED= 74 mg/kg/day	
Rat: infusion of 47.5 mg/kg//h for 1, 2, & 4 hr infusion /day for 14 days; n=5/sex/group (no bolus) TDI= 47.5, 95, &190 mg/kg/day Fospropofol AUC: 96 at high dose Propofol mg/kg/day AUC: 24 HED=30.6 (high dose)	

*Note that propofol was administered as a comparator in the rat (TDI 80 mg/kg, AUC ~7 µg.h/mL) and dog (TDI 51 mg/kg, AUC ~20 µg.h/mL) study and formaldehyde was administered in the monkey (TDI 65 mg/kg, AUC ~24 µg.h/mL).

The Sponsor conducted toxicity studies with bolus intravenous administration of fospropofol disodium followed by a continuous infusion of the test article (≥ 24 hrs) in dog and monkeys and rats (≥ 4 hrs); the procedure was not tolerated in any of the nonclinical species studied. The Sponsor also conducted toxicity studies with the

continuous infusion only of fospropofol disodium in dogs and monkeys up to 6-8 hrs which was well tolerated in both of the species.

Summary of toxicity findings from continuous infusions:

Study*/Number of animals (n)/ Dose (mg/kg) Exposure: Fospropofol /AUC (µg.h/mL) Exposure: Propofol /AUC (µg.h/mL)	**Major Findings**
Monkey: ≥ 24 hrs, continuous infusion; n=3/sex/group TDI= 730 mg/kg/day Fospropofol AUC: 440 Propofol: AUC: 200 HED= 235 mg/kg/day	**Findings from monkey ≥ 24 hrs** **Myocardial degeneration w/neutrophilic infiltration, karyomegaly observed in one animal; Skeletal muscle myofibers w/perimysium, myofibers degeneration/regeneration; Spleen lymphcytosis; Skin neutrophilic arteritis, epidermal necrosis. Active inflammation;** **Findings from dog ≥ 24 hrs** **Inflammation in lungs; Glycogen depletion in liver; Mineral deposits in kidney Thickening of the skin in the injection site;** **Findings from dog ≥ 6 hrs** **Subacute inflammation in kidney** **Findings from monkey≥ 6 hrs:** **No microscopic lesions**
Dog: ≥ 24 hrs, continuous infusion; n= 1/sex/group TDI= 1796 mg/kg/day Fospropofol: ND Propofol: AUC: 245 HED= 997 mg/kg/day	
Monkey: ≥ 8 hrs, (bolus 45+continuous infusion 64) for 8 hrs; n=1/sex/group TDI= 557 mg/kg/day Fospropofol AUC: 516 Propofol: AUC: 216 HED= 185 mg/kg/day	
Dog: ≥ 6 hrs, (bolus 38+continuous infusion 90) for 6 hrs; n=3/smales/group TDI= 458 mg/kg/day Fospropofol AUC: 371 Propofol: AUC: 64 HED= 254 mg/kg/day	

*Note that propofol was administered as a comparator in the monkey ≥ 24 hrs continuous administration (TDI 497 mg/kg/day, HED= 235 mg/kg/day AUC ~187 µg.h/mL) and ≥ 8 hrs continuous administration (TDI 294 mg/kg/day, HED 95 mg/kg/day AUC ~40 µg.h/mL)

The analyses of lung lesions in rat showed perivascular mononuclear cell infiltrates and hyperplastic alveolar epithelial cells within the alveoli. According to the pathologist, the perivascular lesions were formed as a result of intravascular cannulation and infiltration of foreign particles as observed by the presence of hair and skin structures in rats. However, lung inflammation associated with lymphocyte aggregate infiltration in the visceral pleural area was noted in monkeys and dogs in the repeat dose studies also. The histopathological lesions in lungs from the continuous administration of fospropofol disodium showed similar infiltration of alveolar macrophages but were more severe in nature, pleural effusion was noted which might have resulted in cardiac insufficiency noted in these studies. Similar histopathological lesions were noted with the propofol

treated animals, the degree of severity was, however, slightly less than that of fospropofol disodium.

The nature of the lesion in heart causing cardiomyopathy in rat 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 fospropofol disodium administration 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 of fospropofol disodium, however, the severity of the incidence was described as minimal and described as restricted to one or two small focal areas. The toxicokinetics studies in rats were insufficient to interpret the data based on exposure. In the 1-month repeat dose toxicity study in the monkey, lymphocytes aggregates infiltrated in heart, but lesions were not as severe as noted in the rats. In the continuous infusion study in the monkeys, however, 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. Similar histopathological lesions were noted with the propofol treated animals; however, the degree of severity was less than that of fospropofol disodium.

The histological changes in the skeletal muscles were reported in monkeys, in the continuous infusion study, the skeletal muscle lesions are 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 the one month repeat dose toxicity study in the monkey infiltration of lymphocyte aggregates were noted in the skeletal muscles without any further histological lesion. Similar histopathological lesion was noted with the propofol treated animals.

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 in rats. The acute inflammation in liver was associated with mono and polymorphonuclear cell infiltrates in the sinusoids. In the one month repeat dose toxicity study in the monkey infiltration of lymphocyte aggregates were noted in the liver without any further histological lesion. The histological lesion of the liver in the continuous infusion study in dogs consisted of glycogen depletion; however, hepatomegaly was noted in the monkey continuous infusion study. Similar histopathological lesions were noted with the propofol treated animals.

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

The lesions in the spleen were not obvious in the repeat dose studies in dogs and monkeys as observed in rat repeat dose study, however, lesions of similar nature was observed in dogs and monkeys where fospropofol was administered continuously for ≥ 24 hrs. Propofol has been reported to induced corticosteroid production, fospropofol sodium was not tested for the secretion of the adrenal cortical hormones, however, in most of the toxicity studies an increase level of triglycerides were observed which is a marker of the plasma corticosteroid level. Also, in the tissue distribution studies the test article was noted to deposit at high amount in the adrenal cortex indicating a plausible modulation of the adrenal cortical hormones. All these findings indicate that, the test article might have an immunomodulatory effect. In addition parasitic cyst noted in the monkey in an incidence rate higher than control might indicate immunosuppressive effect of the test article in prolonged administration. In general, fospropofol induced hematological changes include decrease in hemoglobin, hematocrit, and RBC in all of the different species studied include decrease in hemoglobin, hematocrit, and RBC which might have resulted from the dilution of the blood resulting from the high volume of the liquid infused during the process of the test article administration. This might be related to the bone marrow cell hyperplasia. In the continuous infusion study (≥ 24 hrs) with the fospropofol disodium in the male dog where the test article exposure was highest, a unique histopathological change was noted in stomach. The lesion consisted of brown pigmented material (moderate), edema, hemorrhage, congestion, venous thrombi, and necrosis suggesting a histamine related effect. Although, histamine release is noted after propofol administration in previous studies, histamine was not examined in this submission either with propofol or with the fospropofol disodium. However, a possibility of histamine release after the test article administration might not be ignored with prolonged continuous administration of the test article.

There was an increased lesion in the injection sites and skin in the test article treated animals in most of the toxicity studies conducted. In the distribution studies increased fospropofol disodium level was noted in the pigmented skin in the rats up to 3-days after a single bolus administration of fospropofol disodium, indicating that the test article is distributed in skin for a long period of time. The lesions in rat 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. The anatomic sites of the lesions were diagnosed in skin-subcutaneous tissues. In the continuous infusion study (≥ 24 hrs) with the fospropofol disodium in the monkey one of the treated animals had full thickness epidermal necrosis of skin accompanied with bacterial contamination and neutrophilic infiltration. Interestingly, a variety of histological lesions in skins such as hemorrhage, chronic inflammation, hyperkeratosis, and squamous cell hyperplasia was noted in increased incidence in the animals treated with fospropofol treated animals compared to those of the controls in the one month repeat dose toxicity study in the monkeys. The injection site reactions were also noted in dogs. In the non clinical studies with propofol submitted with this application similar findings were not reported. The reasons for the skin lesions are not known, biological significance of the findings are yet to be determined.

In summary, the toxicity findings from the fospropofol disodium treated animals as indicated by the histological lesions of lung, liver, kidney, bone marrow, spleen, were observed to be similar in nature compared to those of the propofol treated animals. However, the severity and the incidences of the toxicity in some instances were higher in the fospropofol disodium treated animals than the propofol treated animals may be due to the differences in the exposures of propofol in the plasma. The only differences in the histological lesions between the propofol and the fospropofol disodium treated animals were the skin lesions, the biological relevance of these findings is not known.

Fospropofol disodium was not genotoxic, with or without metabolic activation, in the following assays: Ames bacterial mutation assay and tests for cytogenetic aberrations in vivo in mouse bone marrow lymphocytes. Fospropofol disodium was clastogenic in the mouse lymphoma cell assay in the presence of metabolic activation. Mechanistic study was conducted to determine the cause of clastogenicity. Clastogenicity was not observed in the presence of formaldehyde dehydrogenase indicating that enzymatic digestion of the formate produced by fospropofol in the presence of metabolic activation is required to circumvent clastogenicity.

Fospropofol disodium was evaluated in a complete battery of the reproductive toxicity studies by bolus intravenous administration. The study protocols followed the ICH S5 Guidance, all of the studies are considered valid because maternal toxicity was observed in the maximum tolerated dose.

In the male fertility studies, there was decrease in the sperm count (15%) and sperm density (18%) at high dose (20 mg/kg), based on this finding, the NOAEL for the male fertility is established to be 10 mg/kg (AUC_{inf} for propofol and fospropofol disodium were 357 and 7407 ng.h/mL respectively) by the reviewer. The Sponsor established a NOAEL of 20 mg/kg because the decrease in sperm count is not statistically significant. In the female fertility studies, there were increase in the non viable embryos (2-3 folds) at all doses (5, 10, 20 mg/kg), the finding was observed in all treatment groups. Based on these findings the NOAEL for the female fertility is established to be < 5 mg/kg. The Sponsor believed that the increases in nonviable embryos are not dose related, therefore not treatment related.

Summary of toxicity findings from fertility studies:

Parameters	**Dosages (mg/kg/day)**			
	0	**5 (HED=0.8)**	**10 (HED=1.6)**	**20 (HED=3.2)**
Preimplantation Loss (mean ± sd)	**7.2 ± 7.7**	**9.3 ± 8.6**	**10.2 ± 13.8**	**8.3 ± 8.9**
Nonviable embryos (N)	**7**	**23 (> 3-fold ↑)**	**10 (~ 1.4-fold ↑)**	**15 (> 2-fold ↑)**

Dams w/ any nonviable embryos N (%)	**6 (25)**	**13 (52)**	**7 (28)**	**7 (29)**
% of nonviable embryos/litter (mean ± sd)	**1.9 ± 3.6**	**5.8 ± 6.9 (3-fold ↑)**	**2.4 ± 4.4 (1.3-fold ↑)**	**3.6 ± 6.3 (~2-fold ↑)**
Historical control (% non viable embryo)= 0.8; Range 0.1-1.5				
Caudal sperm count (mean ± sd)	**130 ± 41**	**132 ± 30**	**127 ± 30**	**109 ± 38 (16 % ↓)**
Caudal sperm density (mean ± sd)	**1244 ± 387**	**1195 ± 235**	**1154 ± 247**	**1015 ± 337 (18 %↓)**

In the rat, embryofetal development studies (0, 5, 20, 45 mg/kg), there was an increase in the number of fetus with asymmetric sternal centra and wavy ribs in the treated animals. These variations are believed to be related to the incomplete ossification. In addition, there was an additional central rib in the 7th 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. Increased resorptions of fetus were also noted from all test article treated group. Because of the increase in this incidence compare to that of the control, NOAEL for fetal variations in this study could not be established and was believed to be < 5 mg/kg, ($AUC_{0\text{-}17}$ for fospropofol disodium and propofol were 29 and 8 µg.h/mL respectively).

Summary of embryofetal toxicity findings from rat:

Parameters	**Dose (mg/kg/day)**			
	0	**5 HED=0.8**	**20 HED=3.2**	**45 HED=7.2**
Cervical vertebra (ribs present at 7th cervical vertebra)	1L (4.2%) 1F (0.5%)	3L (13%) 3F (1.7%)	2L (8.0%) 2F (1.0%)	2L (8.7%) 3F (1.1%)
Historical control(%): 0.09; Range 0-4.2				
Ribs /fused, wavy, incomplete ossification	0L (0%) 0F (0%)	0L (0%) 0F (0%)	1L (4.0%) 1F (0.5%)	2L (8.7%) 3F (1.7%)
Historical control (%): 0.66; Range/ litter 0-8.7%, Range/fetus 0-1.2%				
Sternal Centra/ asymmetric, incomplete ossification	0L (0%) 0F (0%)	1L (4.3%) 1F (0.6%)	1L (4.0%) 1F (0.5%)	1L (4.3%) 1F (0.6%)
Historical control (%): 3.99; Range 3.9-4.0				

In the rabbit embryofetal development study (0, 14, 28, 56 mg/kg), there was an increase in the number of fetuses from the test article treated animals than controls with malformations. 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 5th lumbar vertebra and a displaced midline suture in the right nasals. Another fetus from the same litter 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 in the 70 mg/kg/day dose group. The arches of the cervical vertebra were fused in one 28 mg/kg/day fetus. Fused sternal centra were observed in one fetus 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 increased skeletal variations in rabbits were considered not test article related by the Sponsor because they were not dose related. 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 right 12th and 13th right thoracic ribs and short left 11th ribs. The NOEL for the fetal findings was established to be <14 mg/kg/day ($AUC_{0\text{-}18h}$ for fospropofol sodium 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, the variations such as displaced midline suture, angulated hyoids, and wavy ribs are dose related. The gross external alterations are associated with malformations in the skull, vertebrae, and soft tissues and were not observed in the concurrent controls, therefore are considered as test article related.

Summary of embryofetal toxicity findings from rabbit:

Parameter	**Dose (mg/kg/day)**				
	0	**14**	**28 HED=9**	**56 HED=18**	**70 HED=23**
Skull/Irregular Ossification/ (Summarization of all Irregular Ossification: nasal, frontal, palate, parietal)	9L (45%) 13F (8%)	9L (37%) 7F (6%)	11L (58%) 21F (14%)	6L (35%) 14F (12%)	8L (68%) 10F (10%)

Skull/Irregular Ossification/Nasal (Summarization of Internasal suture and Displaced Midline Suture	4L (20%) 5F (3%)	5L (26%) 7F (5%)	5L (26%) 8F (5%)	6L (35%) 10F (8%)	4L (33%) 5F (5.2%)
Skull/ Irregular Ossification: Nasal-Midline Suture Displaced	2L (10%) 2F (1%)	3L (16%) 4F (2.7%)	3L (16%) 5F (3.4%)	3L (18%) 3F (2.5%)	3L (25%) 4F (4.2%)
Hyoid/Angulated	1L (5%) 1F (0.6%)	2L (11%) 3F (2%)	5L (26%) 11F (7%)	2L (12%) 3F (3%)	3L (25%) 5F (3%)

Summary of embryofetal toxicity findings from rabbit contd.:

Parameter	**Dose (mg/kg/day)**				
	0	14	28 **HED=9**	56 **HED=18**	70 **HED=23**
Skeletal Malformation					
Ribs short, fused, wavy 5th-7th	0L (0%) 0F (0%)	0 L (0%) 0F (0%)	0L (0%) 0F (0%)	0L (0%) 0F (0%)	1L (8.3%) 1F (1%)
Thoracic vertebrae Arches small, ribs fused	0L (0%) 0F (0%)	1L (5.3%) 1F (0.7%)	0L (0%) 0F (0%)	0L (0%) 0F (0%)	1L (8.3%) 1F (1%)
Bifid Centrum in the lumber vertebra	0L (0%) 0F (0%)	0L (5.3%) 0F (0.7%)	0L (0%) 0F (0%)	1L (6%) 1F (0.8%)	0L (0%) 0F (0%)
Incompletely ossified palate	0L (0%) 0F (0%)	0L (0%) 0F (0%)	1L (5.3%) 1F (07%)	0L (0%) 0F (0%)	1L (8.3%) 1F (1%)
Skull/ Frontal (Intra-Frontal present)	0L (0%) 0F (0%)	0L (0%) 0F (0%)	1L (5.3%) 1F (07%)	1L (5.9%) 1F (0.8%)	1L (8.3%) 1F (1%)

In the pre and post natal development study in rats (0, 5, 10, and 20 mg/kg), one F_0 dam in the 10 mgs/kg dose group had all litters died at lactation day (LD 2). There was an increase in the number of pups died which between the LDs 1-14. The biological significance of such findings is unknown. The pup mortality between LD 1- 21 was higher in the high dose group animals. In the C-section delivery from F_1 dams, the number of dams with any resorptions increased dose dependently. 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/day).

Summary of embryofetal toxicity findings from rat:

Parameters	Dose (mg/kg/day)			
	0	5 HED=0.8	10 HED=1.6	20 HED=3.2
Mating/Fertility F_0				
Pups found dead; Days 4-14	0.6	0.9	0.4	0.9
Mating/Fertility F1				
Dams w/any resorptions N (%)	9 (41%)	5 (20%)	9 (36%)	13 (54%)
Passive Avoidance Test/Male F1				
Short term memory	20.7 ± 2.6	18.1 ± 19	13.7 ± 11	14.6 ± 15
Long term memory	31.3 ± 24.2	30.7 ± 23	27.3 ± 25	24.2 ± 22

The major findings from the reproductive toxicity studies are test article related increase resorptions of the fetuses, malformation in the fetuses in rabbits, incomplete ossification of ribs in rats and rabbits, and displaced midline suture in the nasal area. The reproductive toxicity studies with propofol also reported increased resorptions and incomplete ossification of bones indicating that the reproductive toxicity findings of fospropofol disodium are related to propofol. The malformations observed in the rabbits and the skeletal variation such as displacement of the mid line suture in the nasal area, however, was not reported in propofol reproductive toxicity studies. The test article induced acidosis, however, was noted in almost all the toxicity studies conducted. Acidosis is well known to induce skeletal anomalies including incomplete ossification. Therefore, test article related changes in the embryofetal development appeared to be related to the secondary pharmacodynamics effect of the test article (Kraut et al 1986, Bernard et al 2005).

It is recommended that fospropofol disodium be labeled as Pregnancy Category 'C', instead of 'B' which is currently suggested by the Sponsor. The reviewer understands that the therapeutic indication is a single dose administration of the product to induce and maintenance short term anesthesia. Therefore the reproductive toxicity studies with repeat dose administration of the product might not be relevant. However, under the current ICH guidelines, the reproductive toxicity studies are valid and did produce reproductive toxicity with fospropofol disodium administration [redacted] b(4)

B. **Pharmacologic activity:** The primary pharmacodynamics of fospropofol disodium is the induction of anesthesia by the production of its active metabolite propofol. Propofol is known as a sedative hypnotic compound which is believed to exert its function primarily by enhancing the activity of the gamma aminobutyric acid (GABA, which is the principal inhibitory neurotransmitter system in the central nervous system) activated chloride channel. The interaction of propofol with the specific membrane

structures decreases the rate of binding of GABA from its receptor, thereby increasing the duration of the GABA activated opening of the chloride ion. It also possesses an ion channel blocking effect in cerebral cortex nicotinic acetylcholine receptors as well as lysophosphatidate signaling in lipid mediator receptor (Chiu et al 2001, Bali et al, 2003).

The drug related activity that is the anesthetic potential of fospropofol disodium was examined and compared with propofol mostly within the toxicity study protocols in rats, rabbits, dogs, and monkeys. In all of the different studies, onset and recovery of anesthesia after fospropofol disodium administration was observed to be delayed compared to that of propofol administration. The degree of anesthesia was also compared after the propofol and the prodrug administration by analyzing different parameters such as voluntary and involuntary movement, flaccid muscle tone, palepebral, pedal, and pupil reflex in different species such as rat, dog, and monkey.

The secondary pharmacodynamics screening assay indicates that propofol antagonized calcium and contractile response (by leukotriene D4, acetylcholine, electrical response, and cholecystokinin) which might potentially cause muscle weakness and might affect passing of food material through the GI tract. Similar secondary pharmacodynamics activity might be expected with fospropofol disodium administration. The safety pharmacology studies demonstrated a decrease in the heart rate and MAP in the animal studies where the test article was administered following the clinical study design.

Fospropofol disodium was adequately examined in vitro and in vivo for understanding its ADME profile. The test article metabolized rapidly to form propofol, phosphate, and formic acid in the presence of alkaline phosphatase in nonclinical species such as rat, dog, and monkey. It 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 by product of the Krebs cycle and the enzyme metabolizing formic acid to formate is present in appreciable amounts in all tissues and it is expected that formic acid formed after fospropofol administration is metabolized rapidly to formate. In the toxicokinetic studies formate levels after fospropofol disodium administrations was observed to be similar to the background levels. The phosphate levels were also assessed in the toxicology studies and were found to remain unchanged after the test article administration.

In vitro and in vivo studies indicate that fospropofol disodium is not a substrate for CYP 450 and therefore CYP enzymes do not play a role in the metabolism of fospropofol disodium. As such, drug-drug interactions with fospropofol disodium due to CYP enzyme effects are anticipated to be minimal.
The AUC and Cmax of after the IV administration of the test article increased dose proportionally in all of the species studies. The in vivo excretion and tissue distribution studies were conducted after IV administration of fospropofol disodium since IV is clinical route of administration. The elimination half life of the test article 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 fospropofol disodium related radioactivity was detected in urine and feces, respectively. A similar elimination profile is expected in human. Fospropofol disodium was observed to be distributed immediately following its administration in the adrenal gland, liver, kidney, bone marrow, salivary gland, thyroid, skin, stomach, and lungs. 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. In summary, the ADME profile of fospropofol disodium is observed to be similar to the non clinical species studied as regards to its absorption, metabolism, and elimination profile. The tissue distributions of the test article in rodents are expected to be similar in primates considering similar protein binding and blood partitioning profile. Wider distribution of the test article in tissues might be expected in human compared to the rodents because increased blood partitioning in primates compared to that of the rodents was noted.

C. Nonclinical safety issues relevant to clinical use:

The current therapeutic indication of fospropofol disodium is short term sedation [redacted] b(4) There is an extensive clinical experience with the active metabolite propofol. The histological lesions observed in lung, heart, kidney, liver, bone marrow, and spleen after the repeated multiple dose (14-28 days) administration of fospropofol disodium was mostly similar to the lesions observed with propofol administration. These lesions were observed in the pivotal studies in the monkeys (HED=53 mg/kg) and dogs (HED=96 mg/kg) w/2x HED and 3x HED respectively indicating that safety margins are narrow for a prolonged administration of the test article in a clinical setting. However, for short term, single dose administration less acute toxicity might be predicted from the nonclinical safety evaluation.

The general toxicity finding related to skin lesions appeared to be unique to fospropofol sodium, based on the toxicity studies conducted with propofol and may be related to the formaldehyde formation. However, the skin lesions were observed only in the repeat dose toxicity studies and therefore its biological relevance in the short term therapeutic indication is not known. In a single dose subcutaneous local toxicity study in rat (100 mg/kg, HED= 16 mg/kg) minimal irritation were noted, it is, however not known, whether the intravenous administration of the compound might induce slight irritation in the skin in human.

The reproductive toxicity findings include resorptions of fetus, malformations, and skeletal variations in rats and rabbits. The reproductive toxicity assessments were also conducted in repeat dose studies according to the ICH Guidelines. The clinical implication of such findings in the short term sedation w/fospropofol sodium is not known, [redacted] b(4) Similarly, the clastogenicity findings observed in the genotoxicity

studies, although observed to be mediated via formaldehyde which is predicted to be metabolized *in vivo* quickly by enzymatic digestion, [redacted] because it is a positive genotoxicity finding and might have effect in prolonged use of the test article and/or overdosing of the test article. b(4)

The fospropofol sodium is recommended to be examined in juvenile animal toxicology studies, prior to its approval in the pediatric population (age from 0-17 years).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

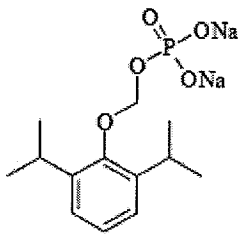
NDA number: 22-244
Review number: 1
Sequence number/date/type of submission: 000/ September 26th, 2007/Original NDA
Information to sponsor: No (x)
Sponsor and/or agent: MGI Pharmaceuticals Inc.
Manufacturer for drug substance: Baxter Pharmaceutical Solutions, LLC
Bloomington, IN, 47403
Reviewer name: Mamata De, Ph.D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: May 30, 2008

Drug:

Trade name: Aquavan (proposed but rejected by the Agency)
Generic name: Fospropofol disodium
Code name: GPI 15715
Chemical name: 1. 2,6-diisopropylphenoxymethyl phosphate, disodium salt
2. Methanol, [2,6-bis(1-methylethyl) phenoxy]-dihydrogen phosphate, disodium salt
3. [2,6-bis(1-methylethyl) phenoxy] methyl disodium phosphate

CAS registry number: 258516-87-9
Molecular formula/molecular weight: $C_{13}H_{19}O_5PNa_2$/332.24

Structure:



Relevant INDs/NDAs/DMFs:

IND#	Name of Drug	Status	Division	Indication	Original Submission Date	Sponsor
62,860	Fospropofol	Active	DAARP	Sedation in the	29-Jun-2001	MGI Pharma

	Injection			[redacted] anesthesia care		

b(4)

DMF#	Subject of DMF	DMF Holder	Submission Date
	[redacted]		

b(4)

Drug class: Anesthetic

Intended clinical population: The intended indication is for the maintenance of short-term anesthesia are adult patients requiring sedation for diagnostic, therapeutics, [redacted]

b(4)

Clinical formulation: Each 30 mL vial of fospropofol contains 35 mg of fospropofol disodium, 2.5 mg of monothioglycerol (MTG 0.25 wt %) and 1.2 mg of TRIS (0.12 wt [redacted]

b(4)

No novel excipients were used in preparation of the drug product formulation, therefore no qualification of excipients were required.

Impurity profile of the test article was reviewed and all of the impurities present in the drug product complied with ICH Q3 A, B and C Guidance.

Route of administration: Fospropofol disodium is intended to use via intravenous bolus injection to induce anesthesia followed by infusion to maintain the anesthesia for a short period of time. [redacted]

b(4)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Study Number	Study Title
Pharmacology	
1-1002929-0	Profiling Screen on Compound GPI 15715 (Biochemical and Radioligand Binding Assays)
1-1002929-1	Pharma Screen on Compound GPI 15715 (CNS - rat, mouse; Cardiovascular - mouse, rat; Respiratory -mouse; GI -mouse; Renal -rat)
1009415	Profiling Screen 2,6-diisopropylphenol (Propofol)

1002944	Pharma Screen 2,6-diisopropylphenol (Propofol)
CDDR-R-953-0101-rar-3	Determination and Evaluation of GPI 15715 Median Hypnotic Dose and Maximum Tolerated Dose in Mice
3000-15715-00-08n	Comparison of Anesthesia Induced by GPI 15715 and Propofol in Sprague-Dawley Rats
CDDR-R-5953-0101-RAR-4	Determination of GPI 15715 Anesthesia Induction and Recovery Times in Rabbits
CDDR-R -5953-0101-RAR-2	GPI 15715 Induced Onset of Anesthesia in a Male Beagle Dog
Safety Pharmacology	
GPI 15715-TOX-04-019:	Effects of GPI 15715 (Fospropofol®), Propofol (Diprivan®) and Propofol (Di-iosopropyl phenol) on Cloned hERG Channels Expressed in Mammalian Cells
GPI 15715-TOX-04-020	Effects of GPI 15715 (Fospropofol®) and Propofol on Action Potentials in Isolated Canine Cardiac Purkinje Fibers
1456-GPI-01	Effects of GPI 15715 (20, 40 and 80 mg/kg, IV) on Blood Pressure, Heart Rate, and ECG in Freely Moving Male Rats
CDDR-R-5953-0101-RAR-1:	The Effect of GPI 15715 on the Cardiovascular System and Renal Sympathetic Nerve Activity in Rabbits
SNAW-110	A Study to Evaluate the Safety and Effects of GPI 15715 Administered to Beagle Dogs
Absorption: Non IV Pharmacokinetic Study	
Absorp\DMPK-06-084	A Pharmacokinetic Study of Single Dose of GPI 15715 Administered by Intravenous Bolus Injection, Subcutaneous Injection, and Oral (Gavage) Routes to Male Rats
Absorp\DMPK-06-102	A Pharmacokinetic Study of Single Dose of FOSPROPOFOL® or DIPRIVAN® Administered by Intravenous Bolus Injection, Gastric Bypass, and Oral (Gavage), Routes to Male Rats
Absorp\04-guil.p01r1&4guilp8	Assessment of the Pharmacokinetics of FOSPROPOFOL (GPI 15715) Following Intravenous, Oral Gavage, Intraduodenal Port, Intrarectal, and Subcutaneous Administration to Male Non-naïve Beagle Dogs
Absorption: Single Dose IV Pharmacokinetic Study	
Absorp\DM-00-011	Preliminary Pharmacokinetics of GPI 15715 in the Rat
Absorp\DM-00-012	Preliminary Pharmacokinetics of GPI 15715 in Dogs
PK-SMP-15715-007	A Toxicokinetic Report: GPI 15715: A Continuous 24-30 Hour Intravenous Infusion Study in Dogs
Absorption: Repeat Dose IV Pharmacokinetic Study	
Absorp\PK-SMP-15715-005a	Toxicokinetic Report: Intravenous Developmental Toxicity Study of GPI 15715 in Rats
Absorp\DM-00-023	Toxicokinetic Report: Fourteen-Day Toxicity Study of GPI 15715 in Sprague-Dawley Rats
Absorp\PK-SMP-006a	Toxicokinetic Report: Intravenous Developmental Toxicity Study of GPI 15715 in Rabbits

Absorp\DM-00-022	Toxicokinetic Report: GPI 15715 and Propofol: A 14-Day Intravenous Infusion Toxicity Study in Dogs
Distribution	
Distrib/6778-152	Tissue Distribution of ^{14}C-GPI 15715 After Administration of an Intravenous Dose to Male Rats
Distrib/6778-145	*In Vitro* Plasma Protein Binding, Protein Binding Interaction, and Blood-to-Plasma Partitioning of [^{14}C]GPI 15715 in Mouse, Rat, Rabbit, Dog, Monkey, and Human
Metabolism	
Metab\dm-00-021	A Preliminary Study of the Metabolic Stability in Mouse, Rat, Dog, and Human Microsomes
Metab\dmpk-06-083	Influence of Time and Temperature on the Metabolism of GPI 15715 by Alkaline Phosphatase
Excretion	
Excr\dm-00-007-snaz-102	Study DM-00-007 (SNAZ-102): Pharmacokinetics and Mass Balance of [^{14}C]GPI 15715 Following Intravenous Administration to Sprague-Dawley Rats
Excr\dm-00-007-snaz-103	Absorption, Excretion, and Pharmacokinetics of [^{14}C]GPI 15715 Following Intravenous Administration to Beagle Dogs
Single Dose Toxicity Study	
Single dose tox\3000-15715-00-04g	Acute Toxicity of GPI 15715 in Sprague-Dawley Rats and CD-1 Mice
Single dose tox\3000-15715-02-01g	GPI 15715 and Formaldehyde: An Acute Intravenous Toxicity Study and Toxicokinetic Study in Cynomolgus Monkeys
Single dose tox\3000-15715-01-02n	Propofol and GPI 15715: A Pilot 8-Hour Anesthesia Study in the Rat
Single dose tox\ [redacted] 458007	A Pilot 6-Hour Infusion Toxicity Study of GPI 15715 (Fospropofol®) in Beagle Dogs
Single dose tox\3000-15715-01-01n	GPI 15715 and Propofol: A Pilot Intravenous Infusion Study in Cynomolgus Monkeys
Repeat Dose Toxicity Study	
Repeat dose tox\3000-15715-00-01n	A Continuous 24-30 Hour Intravenous Infusion Study in Dogs
Repeat dose tox\3000-15715-01-02g	GPI 15715 and Propofol: A 48-Hour Intravenous Infusion Toxicity Study in Cynomolgus Monkeys
Repeat dose tox\3000-15715-00-07g	Fourteen-Day Toxicity Study of GPI 15715 in Sprague-Dawley Rats

b(4)

Repeat dose tox\3000-15715-00-05n	GPI 15715 and Propofol: A 3-Day Range-Finding Intravenous Infusion Toxicity Study in Dogs
Repeat dose tox\3000-15715-00-06g	GPI 15715 and Propofol: A 14-Day Intravenous Infusion Toxicity Study in Dogs
Repeat dose tox\3000-15715-02-02n	GPI 15715: Two-Week Pilot Intravenous Toxicity Study in Cynomolgus Monkeys
Repeat dose tox\3000-15715-03-01g	GPI 15715 and Formaldehyde: Four-Week Intravenous Toxicity Study in Cynomolgus Monkeys
***In vitro* Genotoxicity Study**	
Genetox\42331-in-vitro gpi\ 3000\ 15715-tox-00-03g	*Salmonella-Escherichia Coli* / Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with GPI 15715
Genetox\42331-in-vitro-gpi\3000-15715-tox-00-11g	L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with GPI 15715
Genetox\42331-in-vitro-gpi\15715-tox-04-022	L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with GPI 15715 (FOSPROPOFOL®): Formaldehyde Effects
***In vivo* Genotoxicity Study**	
Genetox \42332-in-vivo\3000-15715-00-12g	*In Vivo* Mouse Micronucleus Assay with GPI 15715
Reproductive and Developmental Toxicity: Fertility and Embryonic Development	
Repro-devp-tox\42351-fert-embryo-devp\3000-15715-01-03g	Intravenous Dosage-Range Developmental Toxicity Study of GPI 15715 in Rats
Repro-devp-tox\42351-fert-embryo-devp\1707-007	Intravenous Fertility and General Reproduction Toxicity Study of GPI 15715 (FOSPROPOFOL®) in Rats
Reproductive and Developmental Toxicity: Embryo-fetal Development	
Repro-devp-tox\42352-embryo-fetal-devp\3000-15715-01-05g	Intravenous Developmental Toxicity Study of GPI 15715 in Rats
Repro-devp-tox\42352-embryo-fetal-devp\3000-15715-01-04g	Study 3000-15715-01-04G: Intravenous Developmental Toxicity Study of GPI 15715 in Rabbits

Reproductive and Developmental Toxicity: Prenatal and Postnatal Development	
Repro-devp-tox\42353-pre-postnatal-devp\1707-006	Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GPI 15715 in Rats, Including a Postnatal Behavioral/Functional Evaluation
Local Tolerance	
Loc-tol\GPI 15715-tox-04-026	Single Dose Toxicity/Irritation Study with GPI 15715 by Subcutaneous Dosing in Sprague-Dawley Rats
Loc-tol\3000-15715-00-09g	Peri-vascular Irritation Study in the Rabbit
Loc-tol\GPI 15715-tox-04-007	Single Dose Vascular Irritation Study of GPI 15715 (Fospropofol®) in Rabbits
Loc-tol\GPI 15715-tox-05-032	A Primary Skin Irritation Study in Rabbits with GPI 15715 (FOSPROPOFOL®)
Loc-tol\GPI 15715-tox-05-031	A Primary Eye Irritation Study in Rabbits with GPI 15715 (FOSPROPOFOL®)
Loc-tol\03t-22169-01	*In Vitro* Hemolysis Study (Modified ASTM – Direct Contact Method): FOSPROPOFOL® Injection 35 mg/mL
Loc-tol\3000\15715-00-10g	*In Vitro* Hemolysis Study (Modified ASTM – Direct Contact Method): GPI 15715
Loc-tol\22737	Hemolysis Test (ASTM Method) Direct Contact Method
Loc-tol\22738	Hemolysis Test (ASTM Method) Direct Contact Method
Loc-tol\22739	Hemolysis Test (ASTM Method) Direct Contact Method
Special Toxicity Study: Antigencity and Oral Toxicology Study	
Other-tox-stud\42371-antigen\GPI 15715-tox-05-033	A Dermal Sensitization Study in Guinea Pigs with GPI 15715 (FOSPROPOFOL®): Standard Buehler Design
Other-tox-stud\42371-antigen\GPI 15715-tox-04-025	7-Day Oral Toxicity Study with GPI 15715 by Daily Gavage in Sprague Dawley Rats

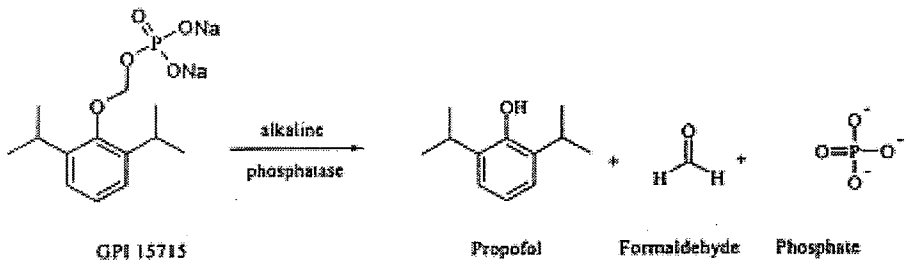
Studies <u>not</u> reviewed within this submission: All submitted studies were reviewed.

Note to reader: Fospropofol disodium was investigated in the pharmacology and toxicity studies under the name GPI 15715, therefore, the name GPI 15715 was used in the following review.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary:

GPI 15715 also known as fospropofol disodium is the O-phosphonomethyl prodrug form of propofol. The chemical structure of propofol was modified with the addition of chemical moiety to make the molecule water soluble. As shown in the table below upon enzymatic digestion the phosphonomethyl group is cleaved to yield propofol the active moiety and the other metabolites, formaldehyde and phosphate. The rationale for developing the aqueous formulation is that the peak concentration of propofol from prodrug would be much lower than its lipid formulation. The hypothesis, if true, will induce a gradual rise of propofol blood level and might be associated with less cardiorespiratory changes than propofol emulsion.



The primary pharmacodynamics of GPI 15715 is the induction of anesthesia by the production of its active metabolite propofol. Propofol is known as a sedative hypnotic compound which is believed to exert its function primarily by enhancing the activity of the gamma aminobutyric acid (GABA, which is the principal inhibitory neurotransmitter system in the central nervous system) activated chloride channel. The interaction of propofol with the specific membrane structures decrease the release rate of binding of GABA from its receptor, thereby increasing the duration of the GABA activated opening of the chloride ion channel (Chiu and White 2001). It also possesses an ion channel blocking effect in cerebral cortex nicotinic acetylcholine receptors as well as lysophosphatidate signaling in lipid mediator receptor. The following model depicting the mechanism of action is reproduced from (Bali and Akabas, 2003).

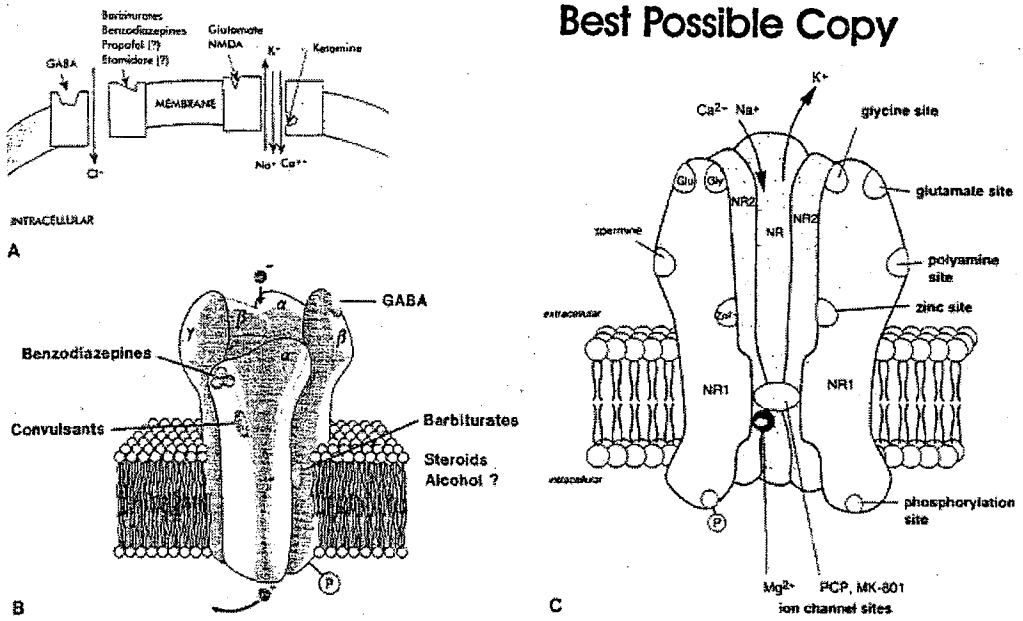


Figure 13-2. (*A*) A model depicting the postsynaptic site of GABA and glutamate within the CNS. GABA decreases the excitability of neurons by its action at the $GABA_A$ receptor complex. When GABA occupies the binding site of this complex, it allows inward flux of chloride ion, resulting in hyperpolarization of the cell and subsequent resistance of the neuron to stimulation by excitatory transmitters. Barbiturates, benzodiazepines, propofol, and etomidate decrease neuronal excitability by enhancing the effect of GABA at this complex, facilitating this inhibitory effect on the postsynaptic cell. Glutamate and its analog *N*-methyl-D-aspartate (NMDA) are excitatory amino acids. When glutamate occupies the binding site on the NMDA subtype of the glutamate receptor, the channel opens and allows Na^+, K^+, and Ca^{2+} to enter or leave the cell. Flux of these ions leads to depolarization of the postsynaptic neuron and initiation of an action potential and activation of other pathways. Ketamine blocks this open channel and prevents further ion flux, thus inhibiting the excitatory response to glutamate. (Reprinted with permission from Van Hemelrijck J, Gonzales JM, White PF: Use of intravenous sedative agents. In Rogers MC, Tinker JH, Covino BG, Longnecker DE (eds): Principles and Practice of Anesthesiology, p 1131. St. Louis, Mosby, 1992.) (*B*) Schematic model of the $GABA_A$ receptor complex illustrating recognition sites for many of the substances that bind to the receptor. (Reprinted with permission from Rochelle D. Schwartz.) (*C*) Model of the NMDA receptor showing sites for antagonist action. Ketamine binds to the site labeled PCP (phencyclidine). The pentameric structure of the receptor, composed of a combination of the subunits NR 1 and NR 2, is illustrated. (Altered with permission from Leeson TD, Iversen LL: The glycine site on the NMDA receptor: Structure–activity relationships and therapeutic potential. J Med Chem 37:4054, 1994.)

Propofol binds to $GABA_A$ receptors and induces its conformational change caused by a disbalance of the five homologous sub units which assembled around the central channel. Each subunit has a 200 amino acid extracellular domain a C-terminal domain with 4 membrane spanning segments (M1, M2, M3, M4). Propofol binding alters GABA receptor structure in the M3 membrane spanning segment region Bali and Akabas (2003). It is, however, not clearly understood how or if propofol binding changes the $GABA_A$ mediated modulation of M1 and M2. Extensive research is ongoing to understand the conformational changes that are induced by the binding of this anesthetic to elucidate the molecular basis of its primary and secondary pharmacological activities.

GPI 15715 and propofol were examined in the radioligand binding screening assay for understanding the specificity of its binding to approximately thirty eight neuronal and peripheral receptors and enzymes. The receptor binding affinity of the test article and propofol was observed to be comparable in this screening assay. Note that neither GPI 15715 nor propofol showed any affinity to GABA agonist or GABA activated chloride channel indicating that the specific ligands used in the binding assays were not the pharmacologically relevant binding sites for either GPI 15715 or propofol.

The pharmacodynamics activity of GPI 15715 was examined and compared with propofol mostly within the toxicity study protocols in rats, rabbits, dogs, and monkeys. In all of the different studies, onset and recovery of anesthesia after GPI 15715 administrations was observed to be delayed compared to propofol. The degree of anesthesia was also compared after propofol and GPI 15715 administration by analyzing different parameters such as voluntary and involuntary movement, flaccid muscle tone, palpebral, pedal, and pupil reflex in different species such as rat, dog, and monkey. The following table provides a comparison of propofol and GPI 15715-induced anesthesia in the cynomolgus monkeys. As noted from this table, the animals were observed to be moderately sedated. All of the sedation parameters were graded comparable between propofol and GPI 15715 suggesting that the depth of anesthesia is similar in all of the species examined.

Cumulative dosage, duration of infusion, and anesthetics parameter in cynomolgus monkeys (reproduced from Sponsor)

	Cumulative Total Dosage (mg/kg)[a]	Duration of Infusion (h)[a]	Number of Observations/Total Number of Observation Intervals						
			Not Responsive	No Voluntary Movement	No Involuntary Movement	Flaccid Muscle Tone	Palpebral Reflex Absent	Pedal Reflex Absent	Pupil Reflex Absent
Fospropofol	121	3.8	2/2	2/2	2/2	0/2	2/2	2/2	1/1
	840	23	6/6	6/6	6/6	2/6	6/6	6/6	2/6
	907	24	6/7	7/7	6/7	2/7	7/7	5/7	5/7
	985	23	5/7	6/7	6/7	1/7	6/7	5/7	5/7
	1930	48	16/18	18/18	16/18	12/18	14/18	16/18	8/18
	2610	48	13/14	14/14	8/14	3/14	13/14	12/14	9/14
Propofol	172	6.4	2/3	2/3	2/3	1/3	2/3	2/3	0/3
	180	6.0	2/2	2/3	0/3	0/2	2/3	2/2	2/2
	227	7.5	3/3	3/3	3/3	0/3	3/3	3/3	2/3
	396	24	6/9	9/9	7/9	2/9	6/9	8/9	4/9
	819	37	12/13	11/13	12/13	10/13	12/13	13/13	9/13
	1090	42	16/17	17/17	14/17	14/17	15/17	17/17	13/17

[a]rounded to 3 and 2 significant figures, for mg/kg and hour, respectively
Source: 3000-15715-01-02G, Text Tables 1, 2 and 3

The Sponsor compared the activity of propofol and GPI 15715 in the *in vitro* and *in vivo* screening assay for CNS, cardiovascular, gastrointestinal, metabolic, allergy, and inflammation. In these pharmacodynamics screening assays propofol demonstrated pharmacologic antagonism for arachidonic acid platelet aggregation, calcium channel type L (ileum), cholecystokinin, chronotropy (right atrium), electrical stimulation (ileum), and leukotriene D_4 (ileum). As expected for a prodrug, GPI 15715 demonstrated no activity ex vivo, where formation of propofol was negligible. However, secondary pharmacodynamics activities similar to propofol is expected with GPI 15715 administration in vivo.

The cardivascular safety assessment of GPI 15715 and propofol was conducted in vitro in the hERG channel assay and in the rabbit Purkinje fiber assay. The test articles were

negative in these assays. In freely moving rats, bolus administration of GPI 15715 produced no changes in the blood pressure and the heart rate. In dogs after a combination of bolus and continuous infusion of GPI 15715 a decrease in the heart rate and mean arterial pressure was noted (MAP).

In summary, the primary pharmacological effects of GPI 15715 as described by the mechanism of action are expected to be identical to its active metabolite, propofol. The drug related activity of GPI 15715 as described by the induction and maintenance of anesthesia is similar to propofol, however, the onset of anesthesia and the recovery time from the anesthesia after GPI 15715 was observed to be delayed compared to that of propofol administration. The reason for the delay is believed to be due to the time required for the conversion of the prodrug to its active metabolite. The secondary pharmacodynamics screening assay indicates that propofol antagonized calcium and contractile response (by leukotriene D4, acetylcholine, electrical response, and cholecystokinin) which might potentially cause muscle weakness and might affect passing of food material through the GI tract. Similar secondary pharmacodynamics activity is expected with GPI 15715 administration. The safety pharmacology studies demonstrated a decrease in the heart rate and MAP in the animal studies where the test article was administered following the clinical study design.

2.6.2.2 Primary pharmacodynamics

Mechanism of action

Study Number: 1-1002929-0
Study Title: Profiling Screen GPI 15715 (Biochemical and Radioligand Binding Assays)
Study Number: 1009415
Study Title: Profiling Screen 2,6-diisopropylphenol (Propofol)

Objective of the study: The objective of the study was to determine the binding activity of GPI 15715 and propofol.
Results: The compounds were screened in a panel of thirty receptors for radio ligand binding at 10 μM concentrations. At this concentration, GPI 15715 did not show 50% binding with any of the receptors tested.
Reviewer's Comments: The receptors tested were as follows:
The percent inhibition of serotonin $5HT_1$, sigma non selective receptor, adrenergic β_1, β_2, and histamine H_1 receptor was 32, 26, 25, 26, and 21 respectively with the compound at 10 μM. The binding experiment with the above mentioned receptors demonstrated that the compound did not bind to any of the receptors by 50%. Therefore, according to the assay criteria the > 20% inhibition of the five receptors mentioned above are non specific. The screening assay for propofol and GPI 15715 did not show any specific binding of the test article under this experimental condition.

Comparison of Receptor Binding activity of Propofol and GPI 15715

Test Number and Title	% Binding

Number	Receptor's Name		
		Propofol	Fospropofol
1	Adenosine A_1	5	2
2	Adenosine A_{2a}	12	-6
3	Adrenergic α_1 nonselective	7	-12
4	Adrenergic α_2 nonselective	-5	5
5	Adrenergic β_1	0	25
6	Adrenergic β_2	42	26
7	Angiotensin A_{T1}	7	14
8	Bradykinin $_{B2}$,	-3	-3
9	Ca Channel $_L$ type	16	-2
10	Dopamine D_1	-7	-5
11	Dopamine D_{2L}	10	0
12	Estrogen ERα	-10	4
13	$GABA_A$ agonist site	-10	14
14	$GABA_A$ chloride channel	-9	6
15	Glucocorticoid	10	-19
16	Glutamate -NMDA	-7	0
17	Glutamate-nonselective	-4	9
18	Glycine strychnine –sensitive	0	4
19	Histamine H_1	-5	21
20	Histamine H_3	20	-16
21	Insulin	1	-19
22	Muscarinic M_1	-6	-6
23	Muscarinic M_2	-6	-9
24	Muscarinic M_3	-14	4
25	Neuropeptide Y_2	3	-8
26	Nicotine acetylcholine	14	-5
27	Opiate δ	-2	11
28	Opiate κ	8	8
29	Opiate μ	6	-9
30	Phorbol ester	12	7
31	Progesterone	15	-1
32	Purinergic P_{2x}	4	-4
33	Serotonin 5-HT_1	12	32
34	Serotonin 5-HT_2	-5	-17
35	Sigma non-selective	22	26
36	Sodium channel site 2	42	1
37	Tachykinin NK_1	11	3
38	Testosterone	-8	-5

Drug activity related to proposed indication

Study Number: Study 3000-15715-00-08n
Study Title: Comparison of Anesthesia Induced by GPI 15715 and Propofol in Sprague-

Dawley Rats

Objective of the study: The objective of the study was to compare the sedation and anesthesia produced by fospropofol and propofol in rat.

Results:

- Induction: 10 and 20 mg/kg propofol caused loss of righting reflex immediately (1/2 rats w/high dose ceased respiring but was revived).
- Induction: 40 and 80 mg/kg fospropofol caused loss of righting reflex in 3 mins.
- Induction: 40 mg/kg propofol caused fatality in 2/2 rats.
- Induction: 160 mg/kg fospropofol caused fatality in 2/2 rats.
- Maintenance: 10 mg/kg bolus along with 20 mg/kg/hr continuous infusion for 30 mins w/propofol and slight increase or decrease of dose for every 15 mins interval keeps the rat sedated for 4 hrs. Fatality occurs in the last hr, ½ rats died, increased triglyceride levels in liver noted. Propofol used in last hr ranged between 15-25 mg/kg/hr.
- Maintenance: 40 mg/kg bolus along with 40 mg/kg/hr continuous infusion for 30 mins w/fospropofol and slight increase or decrease of dose for every 15 mins interval keeps the rat sedated for 4 hrs. No fatality noted, however, cessation 1/2 rats in the last hr, the rat was revived, decreased albumin and alanine transaminase levels in liver noted, fospropofol used in last hr ranged between 0-40 mg/kg/hr

Reviewer's Comments:

The induction of sedation by fospropofol was found to be delayed compared to propofol may be due to the conversion of the prodrug fospropofol to the active moiety the propofol. The induction and maintenance of fospropofol was higher than propofol indicating that propofol is more potent compared to that of fospropofol.

Study Number: Study CDDR-R5953-0101-RAR-3
Study Title: Determination and Evaluation of GPI 15715 Median Hypnotic Dose and Maximum Tolerated Dose in Mice

Objective of the study: The purpose of this study was to determine the MTD and median hypnotic dose (HD_{50}) in mice by fospropofol and compare it to Diprivan™

Results:

- The number of mice got sedated (as described by the loss of righting reflex) were 0/10, 4/10, and 8/10 after 5, 10, and 15 mg/kg of Diprivan™ administration (IV) respectively. Similarly, the number of mice got sedated (as described by the loss of righting reflex) were 0/10, 3/10, 8/10, and 10/10 after 56, 65, 75, and 84 mg/kg of fospropofol administration (IV) respectively.
- The hypnotic dose (HD_{50}) for IV administration of Diprivan™ was determined to be 10.3 mg/kg as established by probit analysis (plotting percent of mice loosing righting reflex for 30 mins). Similarly, the hypnotic dose (HD_{50}) for IV administration of fospropofol was determined to be 68.4 mg/kg as established by probit analysis.
- To determine MTD for mice, 4 mice were administered with 4x HD_{50}, one mouse died, therefore MTD was determined to be 42 mg/kg for Diprivan™. To

determine MTD for mice, 10 mice were administered with 3x HD_{50} i.e., 186 mg/kg, no mice (10 mice tested) died. However with 4x HD_{50}, 280 mg/kg 2/2 mice died, therefore, MTD was determined to be 186 mg/kg for fospropofol.

- The induction time for Diprivan™ was 7 secs w/1.25x HD_{50}, 5.9 secs w/2x HD_{50}; the sleeping time, time between righting and walking, and the time between righting and coordinating at 2x HD_{50} was 4.7, 1, and 3 mins respectively at 2x HD_{50}. The induction time for fospropofol was 105 secs w/1.25x HD_{50}, 81 secs w/2x HD_{50}; the sleeping time, time between righting and walking and the time between righting and coordinating at 2x HD_{50} was 13.4, 2.8, and 7.7 mins respectively at 2x HD_{50} dosage.

Reviewer's Comments:

The HD_{50} for fospropofol was determined to be 3 x higher than that of Diprivan™. The induction and sleeping time for fospropofol is thus longer than that of the Diprivan™, the difference must be due to the time of conversion of fospropofol to its active moiety, propofol.

Study Number: Study CDDR-R5953-0101-RAR-4
Study Title: Determination of GPI 15715 Anesthesia Induction and Recovery Times in Rabbits

Objective of the study: The purpose of this study was to determine the induction time, sleeping time, and walking time following ataxia and time to return to the normal behavior in rabbit by fospropofol and compare it to Diprivan™.

Results:

- The onset of induction by 5, 10, and 15 mg/kg by Diprivan™, (IV administration was approximately 24 sec. The onset of induction by 18, 28, and 38 mg/kg by aquvan (IV administration) was approximately 198 sec.
- The sleeping time, the walking time, and the time when normal motor coordination returned was dose dependent with Diprivan™ and fospropofol. The sleeping time with 5, 10, and 15 mg/kg by Diprivan™, (IV administration) were 118, 317, and 503 secs respectively. The sleeping times with 1, 28, and 38 mg/kg of fospropofol, (IV administration) were 206, 397, and 572 secs respectively.

Reviewer's Comments:

In rabbit the onset of induction by fospropofol was delayed compared to that of Diprivan™. Similarly the sleeping time and recovery was also delayed in fospropofol compared to those of the Diprivan™.

Study Number: Study CDDR-R5953-0101-RAR-2
Study Title: GPI 15715 Induced Onset of Anesthesia in a Male Beagle Dog
Objective of the study: The objective of the study was to compare the onset of sedation after IV administration of fospropofol and Diprivan™.

Results:

- The onset of sedation by propofol (0.067 mmol/kg/min IV; 10 mg/mL) was 40 sec

as described by head drop, the animals slept for 17 min.

- The onset of sedation by fospropofol (0.067 mmol/kg/min,18.6 mg/mL IV, equivalent to 10 mg/kg propofol) was 73 sec as described by head drop, the animals slept for 23 min.
- The onset of sedation by fospropofol (0.157 mmol/kg/min, 37.27 mg/mL IV, equivalent to 20 mg/kg propofol) was 42 sec as described by head drop, the animals slept for 27 min.

Reviewer's Comments:

The onset of sedation was delayed and the sleeping time was prolonged by equimolar concentration of fospropofol compared to that of propofol. However, note that by doubling the infusion time and the dose the onset of sedation was comparable in fospropofol and propofol; in this case the sedation time with fospropofol was prolonged compared to propofol.

2.6.2.3 Secondary pharmacodynamics:

The secondary pharmacodynamics evaluations were studied in the following screening assays.

Secondary Pharmacodynamics
Study Number: 1-1002929-1 **Study Title:** Pharma Screen GPI 15715 (aquavan): CNS –rat, mouse; Cardiovascular - mouse, rat; Respiratory -mouse; GI -mouse; Renal -rat **Study Number:** 100944 **Study Title:** Pharma Screen 2,6-diisopropylphenol (Propofol)
Objective of the study: The objective of the study was to evaluate the maximum tolerated dose (MTD) in mouse and rat after intraperitoneal (IP) and oral (PO) administration of GPI 15715 and propofol. **Results**: Mortality was observed in rat at 100 mg/kg PO, no toxicity was seen in mice at 300 mg/kg PO or 100 mg/kg IP. **Reviewer's Comments**: The Sponsor determined the MTD in rat and mice after different routes of administration. The Sponsor also tested the compound in the following: in vitro and in vivo CNS, cardiovascular, gastrointestinal, metabolic, allergy, and inflammation. The pharmacodynamics screening assays showed that propofol demonstrated activity pharmacologic antagonism for arachidonic acid platelet aggregation, calcium channel type L (ileum), cholecystokinin, chronotropy (right atrium), electrical stimulation (ileum), and leukotriene D_4 (ileum). Oral propofol produced sedation in rats at these dosages. These data suggest that under the conditions of these studies, propofol had pharmacodynamic activity. As expected for a prodrug, GPI 15715 demonstrated no activity ex vivo, where formation of propofol was negligible. The most likely reason that there was no significant blood pressure and heart rate effect in the rat following oral administration of fospropofol was that the dosage and route were suboptimal.

Test Number and Title		Propofol	Fospropofol
Maximum Tolerated dose in Mice			
1	Maximum tolerated dose, autonomic sign 1 hr, with 10, 100, 300 mg/kg PO	100 mg/kg	NR
2	Maximum tolerated dose, autonomic sign 1 hr with 30, 100 mg/kg IP	100 mg/kg	NR
3	Maximum tolerated dose, autonomic sign 1,2, and 3 days either with 10, 100, 300 mg/kg PO or with 30 and 100 mg/kg IP	100 mg/kg	NR
Modulation of Central Nervous System Using Following Tests			
1	Analgesia by PQ writhing in mice after 100 mg/kg PO administration	11% R Criteria ≥ 50%	16% R Criteria ≥ 50%
2	Analgesia by tail flick in mice after 30 mg/kg IP administration	6 % Criteria ≥ 50%	0% Criteria ≥ 50%
3	Anxiety by 5-MEODMT potentiation in rat after 30 mg/kg IP administration	0 Criteria ≥ 2 of 3	1 R Criteria ≥ 2 of 3
4	Cholinergic agonism (central and peripheral) in rat (PO) after the administration of 10 and 100 mg/kg respectively.	0 Criteria ≥ 2	0 Criteria ≥ 2
5	Cholinergic antagonism (central and peripheral) in mouse (PO) after the administration of 100 mg/kg respectively.	4 AN Criteria ≤3	6 AN Criteria ≤3
6	Convulsion in mouse (PO, 100 mg/kg) after electric shock	3R Criteria ≤1of 3	3R Criteria ≤1of 3
7	Convulsion in mouse (PO, 100 mg/kg) after metrazole administration	0 Criteria ≥ 4	2R Criteria ≥ 4
8	Depression, behavioral test in mouse (PO, 300 mg/kg)	45 Criteria < 40	58R Criteria < 40
9	Depression, tetrabenazine induced hypothermia in mouse (PO, 30 mg/kg)	2% Criteria ≥ 50%	0% Criteria ≥ 50%
10	Dopamine agonism /antagonism in mouse (IP, 30 mg/kg)	0% Criteria ≥ 3/50%	0% Criteria ≥ 3/50%
11	Motor coordination, rotarod in mouse (po, 100 mg/kg)	3R Criteria ≤ 1of 3	3R Criteria ≤ 1of 3
12	Motor stimulation in mouse (300 mg/kg)	0 Criteria > 12	0 Criteria > 12
***Ex vivo* Modulation of Cardiovascular System (30 μM)**			
1	Adenosine A_1, α_{1A}, α_{2A} binding in the isolated vas deferens in rat	α_{1A} 18% Criteria ≥ 50%	0% Criteria ≥ 50%
2	Adrenergic β_1 binding in the isolated left atria in guinea pig	21% Criteria ≥ 50%	0% Criteria ≥ 50%
3	Angiotensin 1 binding in the isolated ileum in guinea pig	36% AN Criteria ≥ 50%	14% AN Criteria ≥ 50%

4	Arachidonic Acid evaluation in the platelet rich plasma in rabbit	100% Criteria ≥ 50%	0% Criteria ≥ 50%
5	Calcium channel Type L binding in the isolated ileum in guinea pig	91% AN Criteria ≥ 50%	22% AN Criteria ≥ 50%
6	Cardiac ionotropy in left atria in guinea pig	-16%R Criteria ≥ 50%	-25%R Criteria ≥ 50%
7	Chronotropy in right atria in guinea pig	28% AN Criteria ≥ 50%	38% AN Criteria ≥ 50%
8	Contractility in the vas deferens in rat	0% Criteria ≥ 50%	0% Criteria ≥ 50%
9	Depolarization of the portal vein in rat	16% AN Criteria ≥ 50%	31% AN Criteria ≥ 50%
10	Spontaneously activated portal vein in rat	22% R Criteria ≥ 50%	25% R Criteria ≥ 50%
11	Thromboxane A2 binding in the platelet rich plasma in vitro in rabbit	0% Criteria ≥ 50%	0% Criteria ≥ 50%
***In vivo* Modulation of Cardiovascular System (100 mg/kg)**			
1	Cardiac arrhythmia in mice, chloroform induced (IP)	3R Criteria ≤ 1of 3	Criteria ≤ 1of 3
2	Cardiovascular, blood pressure and heart rate (1,2, 4 hr) in rat PO (5 experiments)	-15% Criteria ≥ 20%	-15% Criteria ≥ 20%
3	Hypoxia in mice, hypobaric (IP)	-1% Criteria ≥ 100%	0% Criteria ≥ 100%
4	Hypoxia, in mice, KCN induced (PO)	0% Criteria ≥ 50%	0% Criteria ≥ 50%
Modulation of Metabolism			
1	Diet induced total cholesterol in mice (PO, 100 mg/kg)	0% Criteria ≥ 20%	9% Criteria ≥ 20%
2	Serum glucose in mice (PO,100 mg/kg)	7% Criteria ≥ 40%	0% Criteria ≥ 40%
3	Renal function: in rat: Kaluersis, Saluresis, Urine volume increase and decrease (PO,30 mg/kg)	111-137% Criteria ≥ 50-200%	93-122% Criteria ≥ 50-200%
***In vitro* Modulation of Parameters Related to Allergy and Inflammation (30 μM)**			
1	Bradykinin B2 binding in the ileum in guinea pig	25 % AN Criteria ≥ 50%	5 % AN Criteria ≥ 50%
2	Tracheal contractility in guinea pig	40% R Criteria ≥ 50%	0% Criteria ≥ 50%
3	Leukotriene D4 binding in the ileum in guinea pig	58% AN Criteria ≥ 50%	14% AN Criteria ≥ 50%
4	PAF platelet aggregation in vitro	4% AN Criteria ≥ 50%	2% AN Criteria ≥ 50%
5	Tachykinin NK_1 binding in the ileum in guinea pig	47% AN Criteria ≥ 50%	3% AN Criteria ≥ 50%

***In vivo* Modulation of Parameters Related to Allergy and Inflammation**			
1	Cutaneous anaphylaxis in rat (PO,100 mg/kg)	0% Criteria ≥ 50%	0% Criteria ≥ 50%
2	Histamine H1 antagonism in rat (PO,100 mg/kg)	0% Criteria ≥ 50%	0% Criteria ≥ 50%
3	Inflammation induced by carrageen (PO,100 mg/kg)	4% Criteria ≥ 30%	4% Criteria ≥ 30%
***In vitro* Modulation of Parameters Related to Gastrointestinal Function (30 µM)**			
1	Cholecystokinin CCK_A binding in ileum in guinea pig	64%AN Criteria ≥ 50%	0% Criteria ≥ 50%
2	Decrease in electric stimulation in ileum in guinea pig	52%R Criteria ≥ 50%	20% Criteria ≥ 50%
3	Increase in electric stimulation in ileum in guinea pig	0% Criteria ≥ 50%	0% Criteria ≥ 50%
4	Electrical stimulation spasm in ileum of guinea pig	0% Criteria ≥ 50%	0% Criteria ≥ 50%
***In vivo* Modulation of Parameters Related to Gastrointestinal Function**			
	Cholinergic antagonism in mouse (PO ,100 mg/kg)	6AN Criteria ≤ 3	6AN Criteria ≤ 3
	Gastric Acidity in rat (IP, 10 mg/kg)	3% Criteria ≥ 50%	0% Criteria ≥ 50%
	Gastric ulcers in rat induced by ethanol (PO, 10, 100 mg/kg)	0% Criteria ≥ 50%	25% Criteria ≥ 50%
	Serotonin 5-HT_3 in mouse (IP, 10 mg/kg)	38% Criteria ≥ 50%	0% Criteria ≥ 50%

2.6.2.4 Safety pharmacology

Cardiovascular effects

Study Title: Effects of GPI 15715 (Fospropofol®), Propofol (Diprivan®) and Propofol (Di-iosopropyl phenol) on Cloned hERG Channels Expressed in Mammalian Cells

Study number: GPI 15715-TOX-04-019

Study design: The hERG channel activation by propofol, diprivan (emulsion solution), and GPI 15715 was examined in human kidney cell (HEK 293).

Results & Reviewer's Comments: GPI 15715 inhibited the hERG channel by 7% only at 3000 µM indicating that IC_{50} is >3000 µM and the inhibition is not physiologically relevant. Diprivan inhibited the hERG channel by 38% at 300 µM; however, due to its lipid based formulation, it induced significant leak current which interfered with recoding

and therefore the data is inconclusive. So, propofol itself was tested at different concentrations. At propofol concentrations of 30, 100, 200, and 300 μM approximately 15, 49, 82, and 96% hERG channel inhibition was noted respectively indicating an IC_{50} of 92.8 μM.

The study was conducted in the [redacted]. under GLP conditions. The active ingredient of GPI 151715, propofol has an IC_{50} in a μM for hERG channel inhibition, suggesting that its effect on Ikr, the rapidly activating, delayed rectifier of the cardiac potassium current may occur only at very high concentrations. b(4)

Study Title: Effects of GPI 15715 (Fospropofol®) and Propofol on Action Potentials in Isolated Canine Cardiac Purkinje Fibers

Study number: GPI 15715-TOX-04-020

Study design: The study was designed to determine the effect of GPI 15715 and its metabolite propofol on the action potential of isolated canine Purkinje fibers.

Result & Reviewer's Comments: The study was conducted in [redacted] under GLP conditions. The propofol at concentrations of 30, 100, and 300 μM were added to four fiber preparations at 3 stimulus intervals of basic cycle lengths 2, 1, and 0.5 secs. There were no changes in the resting membrane potential, maximum rate of depolarization, and amplitude of the action potential. Similarly, GPI 15715 at 10 fold higher concentration than that of propofol (300, 1000, and 3000 μM) did not change any of the parameters responsible for the prolongation of Q and T waves. There were shortening of APD 60 and APD 90 by both of these compounds, the significance of these findings, however, are not known. b(4)

Study Title: Effects of GPI 15715 (20, 40 and 80 mg/kg, IV) on Blood Pressure, Heart Rate, and ECG in Freely Moving Male Rats.

Study number: 1456-GPI-01

Study design: The study was designed to determine the cardiovascular effect of fospropofol in non anesthetized rats and to compare these effects of fospropofol with that of propofol.

Results and Reviewer's Comments: The study was conducted under GLP and quality assurance certification was submitted with this study report. Saline was used as the vehicle for the intravenous administration of propofol and fospropofol. The dose used for propofol was 5, 10, and 15 mg/kg and the dose used for fospropofol was 20, 40, and 80 mg/kg. The parameters studied for the evaluation of the cardiovascular safety was heart rate, ECG, and systolic and diastolic blood pressure. As shown in the Sponsor's figure # 1 and 5 below, there were no statistically significant differences in the heart rate after the vehicle, propofol, and the fospropofol administration. Similarly no changes in the ECG

compared to vehicle were observed either with propofol or with fospropofol. As regards to the parameters such as mean blood pressure (MBP) and systolic and diastolic pressure no meaningful changes were noted with at 10 and 15 mg/kg and fospropofol at 20 and 40 mg/kg (refer to fig 3, 4, 7, and 8). A decrease in the MBP, however, was noted with 80 mg/kg of fospropofol compared to the vehicle control up to 45 mins of the test article administration. The MBP w/80 mg/kg fospropofol at 0, 15, 30, 45, 60 mins, and 6 hrs were 131, 95, 93, 98, 106, and 104 mmHg respectively and that after the vehicle administration at the same time points were saline 132, 122,119,118, 115, and 104 mmHg respectively.

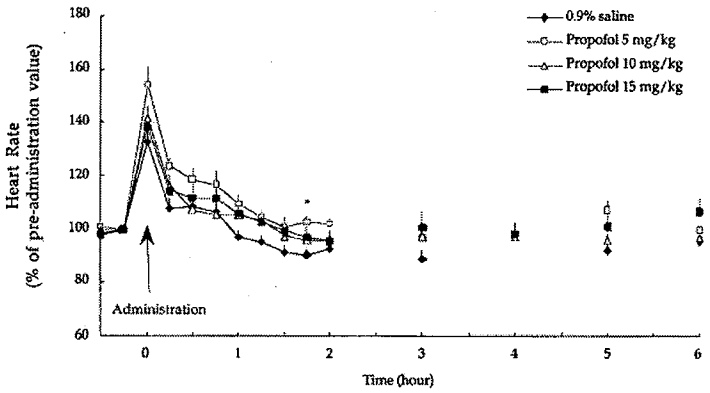


Figure 1: Effect of reference item (propofol) administered *i.v.* on heart rate variations in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.9% saline) or reference item was done immediately before time 0. * $p<0.05$ versus vehicle.

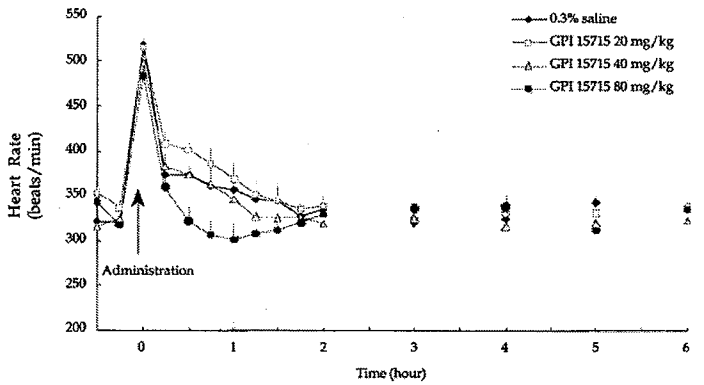


Figure 5: Effect of test item (GPI 15715) administered *i.v.* on heart rate in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.3% saline) or test item was done immediately before time 0.

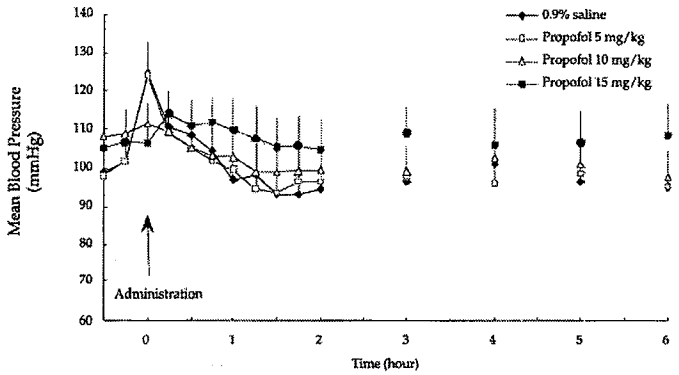


Figure 2: Effect of reference item (propofol) administered *i.v.* on mean blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.9% saline) or reference item was done immediately before time 0.

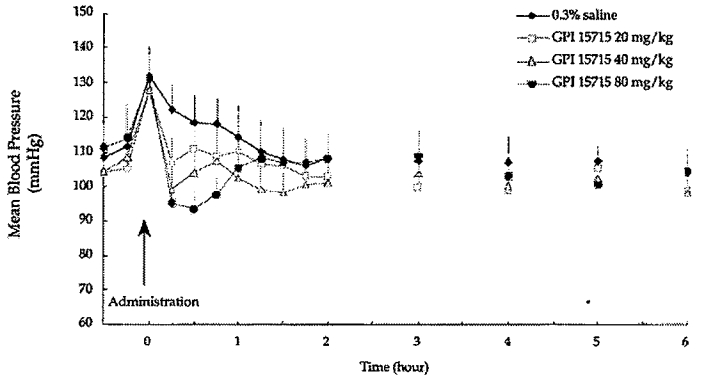


Figure 6: Effect of test item (GPI 15715) administered *i.v.* on mean blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.3% saline) or test item was done immediately before time 0.

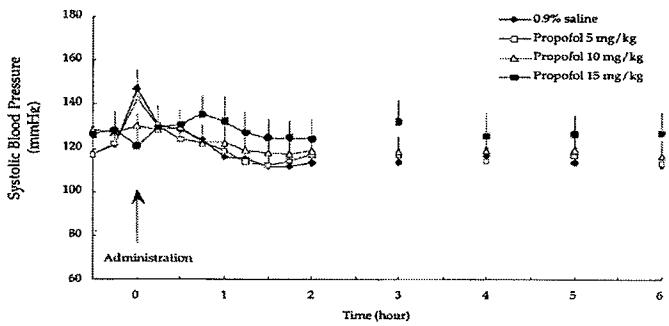


Figure 3: Effect of reference item (propofol) administered *i.v.* on systolic blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.9% saline) or reference item was

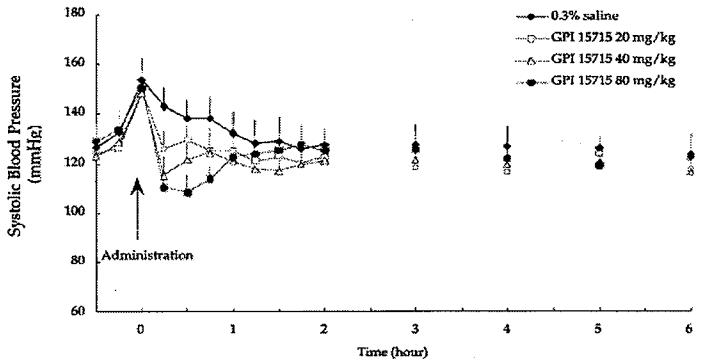


Figure 7 : Effect of test item (GPI 15715) administered *i.v.* on systolic blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.3% saline) or test item was done immediately before time 0.

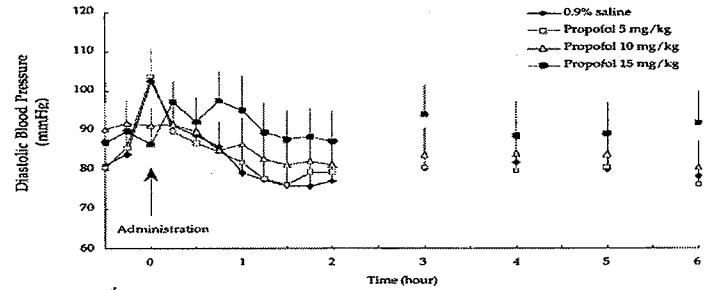


Figure 4 : Effect of reference item (propofol) administered *i.v.* on diastolic blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.9% saline) or reference item was done immediately before time 0.

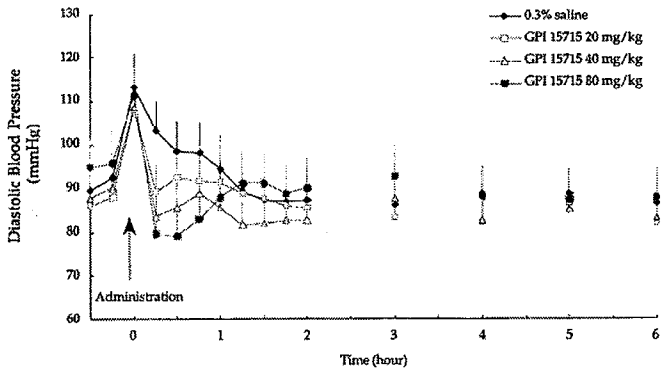


Figure 8 Effect of test item (GPI 15715) administered *i.v.* on diastolic blood pressure in male Sprague-Dawley rats. Values are means ± SEMs of 8 determinations. The administration of vehicle (0.3% saline) or test item was done immediately before time 0.

Study Title: The Effect of GPI 15715 on the Cardiovascular System and Renal Sympathetic Nerve Activity in Rabbits

Study number: CDDR-R-5953-0101-RAR-1

Study design: The study was designed to assess the MAP and renal sympathetic nerve activity (RSNA), phrenic nerve activity (PNA), and respiratory frequency in rabbits (n=8) after Diprivan and GPI 15715 administration

Results and Reviewer's Comments: The MAP and heart rate in rabbits treated either with GPI 15715 or Diprivan decreased dose dependently. The degree of the changes in the cardiac parameters was comparable with both of the compounds. A biphasic change in renal sympathetic nerve activation (RSNA) was observed after the administration of Diprivan; the RSNA increased 140% of baseline and then decreasing up to 90% at 3 mins. Similar changes in the RSNA were noted after the administration of GPI 15715; the RSNA increased 120% of baseline and then decreasing to the baseline level at 1.5 mins.

Under the denervated condition the decrease of PNA was 76% at 4 min post dosing with Diprivan; no such changes were noted after the administration of GPI 15715. The respiratory frequency (RF) decreased at 30 secs post dosing; the decrease after Diprivan and GPI 15715 administration was 38 and 80% of the base line respectively.

The study was conducted in [redacted] The decrease in MAP and HR in rabbit as noted after GPI 15715 administrations is also noted in all of the species studied. The current study also indicates that the degree of anesthesia and the kinetics of the anesthesia as determined by sympathetic nerve activation/reflex, RF and PNA is different after the administration of GPI 15715 than that of Diprivan and therefore the secondary pharmacodynamics effect after GPI 15715 administration would expected to be different than that of Diprivan administration. b(4)

Study Title: A Study to Evaluate the Safety and Effects of GPI 15715 Administered to Beagle Dogs

Study number: SNAW-110

Study design: To evaluate the cardiovascular, neurological, and pharmacodynamics effects of GPI 15715 in beagle dogs (3/sex/group) via bolus and intravenous infusion with different doses. The effect of GPI 15715 was compared to Diprivan by using equimolar concentration of propofol. The Sponsor's study design table is reproduced below. The cardiovascular parameters such as MAP and HR were examined. The neurological changes were assessed through EEG analysis.

Text Table 1 summarizes the study design. A comprehensive listing of actual doses is presented in Table 1.

Text Table 1
Study Design

Group	No. of Animals	Session	Substance	Dose Level (mg/kg)	Dose Concentration	Dosing Paradigm	Observation Period
1	3 Males/ 3 Females	1	Diprivan®	DTE [a], BSI [b]	10 mg/mL	Bolus+Inf [c, d]	To ~10 min after regaining consciousness
		2	GPI 15715	DTE, BSI	20 mg/mL	Bolus+Inf [e, d]	
		3		DTE, Sed [f]		Bolus+Inf [g, d]	
		4		40 mg/kg		Bolus	Up to 2 hours Post Administration
		5		40 mg/kg		10 min Infusion	
		6		15 mg/kg		10 min Infusion	

[a] DTE – Dose to effect
[b] BSI – Burst suppression for 1 second (EEG: approximately 1 hertz median frequency)
[c] Starting doses, Session 1
Males: 1001 = 8 mg/kg + 24 mg/kg/hr; 1002 = 10.7 mg/kg + 32 mg/kg/hr;
1003 = 13.4 mg/kg + 40 mg/kg/hr
Females: 1101 = 16.1 mg/kg + 48 mg/kg/hr; 1102 = 16.1 mg/kg + 48 mg/kg/hr;
1103 = 18.8 mg/kg + 56 mg/kg/hr
[d] If effect was not obtained in 15 minutes after start of infusion, the infusion rate was increased 33% of the original dose level every 15 minutes until effect was achieved.
[e] Starting doses, Session 2
Males: 1001 = 27 mg/kg + 60 mg/kg/hr; 1002 = 27 mg/kg + 60 mg/kg/hr;
1003 = 20 mg/kg + 45 mg/kg/hr
Females: 1101 = 34 mg/kg + 75 mg/kg/hr; 1102 = 34 mg/kg + 75 mg/kg/hr;
1103 = 41 mg/kg + 90 mg/kg/hr
[f] Sed – Sedation (EEG: approximately 4 hertz median frequency)
[g] Starting doses, Session 3
41 mg/kg + 120 mg/kg/hr

Results and Reviewer's Comments: There was a decrease in the MAP and HR in the beagle dogs following the test article administration. Similar changes were noted after Diprivan administration. EEG analyses revealed comparable changes in the voltage suppression but not in the burst suppression indicating that the changes observed are consistent with moderate anesthesia.

2.6.2.5 Pharmacodynamics drug interactions:

There were no pharmacodynamics drug interaction studies submitted with this application.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS

2.6.4.1 Brief summary

The pharmacokinetics (PK) profile of GPI 15715 was evaluated after single dose studies in different species such as rats and dogs. The pharmacokinetic analyses in the Sprague Dawley rats (Study #s DMPK 06-084 and DMPK 06-124) were conducted following different route of administrations such as intravenous (5, 10, 25, 50, and 75 mg/kg), subcutaneous (25, 50, 75, and 100 mg/kg), oral (20, 50, and 100 mg/kg), and gastric bypass (1, 3, 10, 30, and 100 mg/kg). The absolute bioavailability of fospropofol disodium following subcutaneous (SC), oral, and gastric bypass administrations ranged between 126 to 180%, 0.448 to 3.46%, and 0.811 to 3.29%, respectively. The higher bioavailability of fospropofol disodium following SC delivery doses is likely due to a lower rate of metabolism compared with IV doses. The pharmacokinetic analyses were also conducted in the beagle dogs (Study #s 04-GUIL P01R1 & 4GUIL P8) following different routes of administrations with a single administration of 16 mg/kg. Based on AUC, GPI 15715 exposures in dogs were highest after subcutaneous administration followed by (in decreasing order) IV/intrarectal/oral/intraduodenal administrations. In two other pharmacokinetic studies in rat (DM00-011) and dog (DM00-12) the PK profile of GPI 15715 was analyzed after a single intravenous (10 and 7 mg/kg in rat and dog respectively) administration. A difference was noted between the two species; in rat GPI 15715 was distributed biexponentially, the half life for two phases were 2.3 and 19.4 mins, similarly propofol derived from the GPI 15715 was also noted to be distributed biexponentially and the half life for two phases were 2.9 and 133 mins. In dog, biexponentially distribution was not noted and half lives were 7.21 and 1.27 L/min for GPI 15715 and propofol derived form GPI 15715 respectively.

The distribution study (Study # 6778-145) was conducted after ^{14}C GPI 15715 administration in the Long Evans rats. The maximum radioactivity was located in all tissues immediately after the intravenous administration of the test article except fat and intestine where GPI 15715 was noted to be at higher concentration at 1 hr and between 3-8 hrs respectively. The distribution in the intestine may indicate elimination route of the test article. The biological significance of the accumulation of the compound in fat at 1 hr is not known, the concentration of GPI 15715 in fat, however, decreased by 72 hrs. The maximum concentration of 9.95 µg-equivalents/g of radioactivity was detected in brain at 0.083 h post dose and steadily declined to 0.0931 µg-equivalents/g by 120 h. The presence of radioactivity in the brain indicates that the ^{14}C-fospropofol-derived radioactivity crosses the blood-brain barrier. The higher radioactivity concentrations at after 5-day post dosing (in decreasing order) were found in the following tissues: adrenals, liver, kidney, bone marrow, salivary glands, thyroid, and lungs. GPI 15715 was found to be highly protein bound (92.8-97.3) in a protein binding study (Study # 6778-152) at concentrations between 0.5-100 µg/mL in all of the species studied (rat, mouse, rabbit, dog, monkey and human).

The metabolic stability (Study # DM-00-021) of GPI 15715 was studied in vitro in mouse, rat, dog, and human microsomes. In the presence and the absence of NADPH, the stability over a 2-hr period in mouse, rat, dog, and human were 72, 52, 19, and 65% respectively indicating CYP 450 independent metabolism probably due to the enzymatic digestion by alkaline phosphatase. The in vitro enzyme induction study (Study # DMPK 06-083) with GPI 15715 indicate that in the presence of the alkaline phosphatase enzyme

at 37°C, 2/3 of GPI 15715 was converted to propofol and the conversion was complete by 20 mins indicating that the enzyme induction was not dependent on the concentration of the substrate or the product. The Sponsor also collected the livers from the saline, propofol and GPI 15715 treated animals from the 14-day toxicity (Study # 3000-15751-00-06g) study in dog to evaluate the enzyme induction and inhibition by CYP 450 and total protein. No differences were noted in the total protein and CYP 450 content between the different groups indicating minimal possibility of CYP mediated metabolism of the test article.

The excretion of ^{14}C GPI 15715 was determined in the mass balance study in Sprague Dawley rats (DM00-007) and dogs (DM00-008). The primary route of excretion was urinary for both males (64.6% of the dose) and females (76.6% of the dose). Fecal elimination accounted for 14.9% and 10.0% for males and females, respectively. The total of cage debris, rinses, wipes and wash accounted for 6.33% and 4.34% for males and females, respectively. The overall recovery of radioactivity was approximately 86% for male and approximately 91% for female dogs. By 48 h, the excretion of radioactivity was almost complete. In rat the excretory pattern of GPI 15715 was almost similar to that observed in dogs. The primary route of excretion was urinary for both males (65.7% of the dose) and females (76.3% of the dose). Fecal elimination accounted for 21.9 % and 10.4% of the total dose excreted for males and females, respectively. The cage rinses and wash accounted for approximately 2% of the dose, and there was <2% of the dose remaining in the carcasses. The overall total recovery of radioactivity was approximately 91%. By 48 h, the excretion of radioactivity was almost complete.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

Study title: A Pharmacokinetic Study of Single Dose of GPI 15715 Administered by Intravenous Bolus Injection, Subcutaneous Injection and Oral (Gavage) Routes to Male Rats

Study number: Absorp\DMPK-06-084

Study design: Male rats (n=12) were administered with intravenous, oral, or subcutaneous administration of GPI 15715. The dosages administered were described in the following study design table reproduced from the Sponsor's submission.

Table A: Experimental Design

Route of Administration	Number of Animals	Gender	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)[a]
IV	12	Male	GPI 15715	10	2.0	5.0
	12	Male	GPI 15715	25	2.0	12.5
	12[b]	Male	GPI 15715	50	2.0	25.0
	12	Male	GPI 15715	75	2.0	37.5
SC	11	Male	GPI 15715	25	2.0	12.5
	12	Male	GPI 15715	50	2.0	25.0
	12	Male	GPI 15715	75	2.0	37.5
	12	Male	GPI 15715	100	2.0	50.0
PO	6	Male	GPI 15715	100	2.0	50.0

[a] Prepared in 0.9% saline

[b] One of the 12 Rats (#10) died during study procedure

Results and Reviewer's Comments: As indicated in the following table and figure reproduced from the Sponsor's submission, there was a dose related increase in the Cmax and AUC of GPI 15715 and its metabolite propofol after a single oral, subcutaneous, or intravenous administration of the test article. The elimination half life ranged between 0.55-3.41 hrs, 0.76-3.36 hrs, and 0.31-1.53 hrs after oral, subcutaneous, and intravenous administration respectively. Oral bioavailability was lower than that of the subcutaneous bioavailability. The absolute bioavailability of propofol after intravenous administration was > than 82%.

Table 29. Individual and Mean Pharmacokinetic Parameters of GPI 15715 and Propofol following administration of a single oral administration of GPI 15715 to male Rats

Analyte	Rat	Dose (mg/kg)	T_{max}[†] (min)	C_{max} (µg/mL)	AUC_{0-t} (hr•µg/mL)	$AUC_{0-\infty}$ (hr•µg/mL)	$T_{1/2}$ (hr)
GPI 15715	1	100	30.0	0.284	0.684	NC	NC
	2	100	15.0	0.431	0.276	0.295	0.490
	3	100	15.0	0.441	0.207	0.228	0.630
	4	100	15.0	1.64	0.647	0.652	0.730
	5	100	15.0	5.36	2.43	NC	NC
	6	100	15.0	1.59	0.653	0.664	0.350
		N	6	6	6	4	4
		Mean	15.0	1.62	0.817	0.460	0.550
		SD	(15.0-30.0)	1.93	0.819	0.231	0.160
Propofol	1*	100	NC	NC	NC	NC	NC
	2*	100	NC	NC	NC	NC	NC
	3	100	480	0.0389	0.216	NC	NC
	4	100	120	0.0609	0.245	0.303	2.86
	5	100	15.0	0.187	0.440	0.571	4.38
	6	100	15.0	0.0431	0.0777	0.124	3.00
		N	4	4	4	3	3
		Mean	67.5	0.0824	0.245	0.333	3.41
		SD	(15.0-480)	0.0703	0.149	0.225	0.840

[†] Median (range)
NC: not calculated
* Not used for summary statistics due to too few samples.

Figure 9: GPI 15715 C_{max} vs. Dose (mg/kg) Following Administration of a Single Intravenous Injection Dose of 10, 25, 50, and 75 mg/kg GPI 15715 and a Single Subcutaneous Injection Dose of 25, 50, 75, and 100 mg/kg GPI 15715 to Male Rats

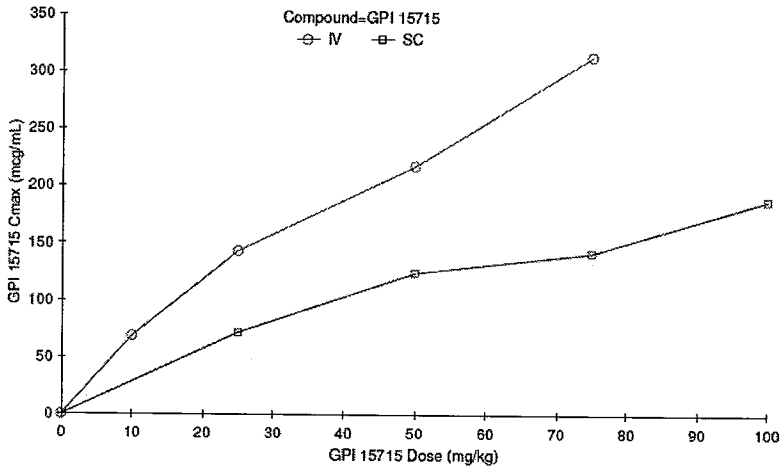


Figure 10: GPI 15715 $AUC_{(0-t)}$ vs. Dose (mg/kg) Following Administration of a Single Intravenous Injection Dose of 10, 25, 50, and 75 mg/kg GPI 15715 and a Single Subcutaneous Injection Dose of 25, 50, 75, and 100 mg/kg GPI 15715 to Male Rats

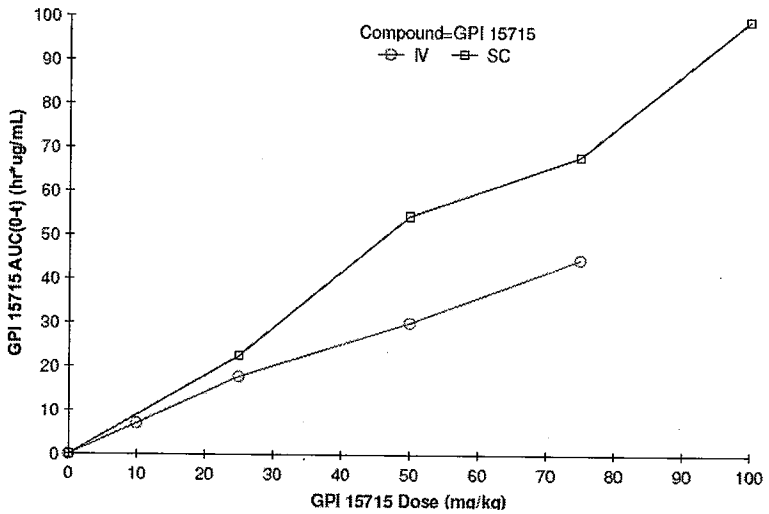


Figure 11: Propofol Cmax vs. GPI 15715 Dose (mg/kg) Following Administration of a Single Intravenous Injection Dose of 10, 25, 50, and 75 mg/kg GPI 15715 and a Single Subcutaneous Injection Dose of 25, 50, 75, and 100 mg/kg GPI 15715 to Male Rats

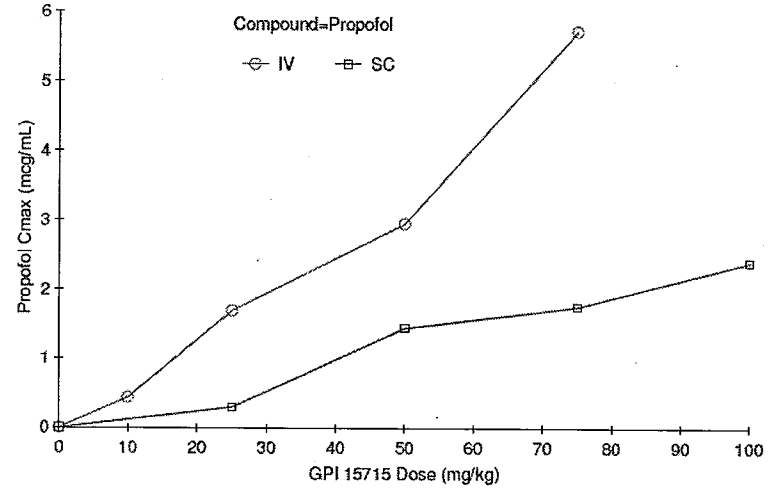
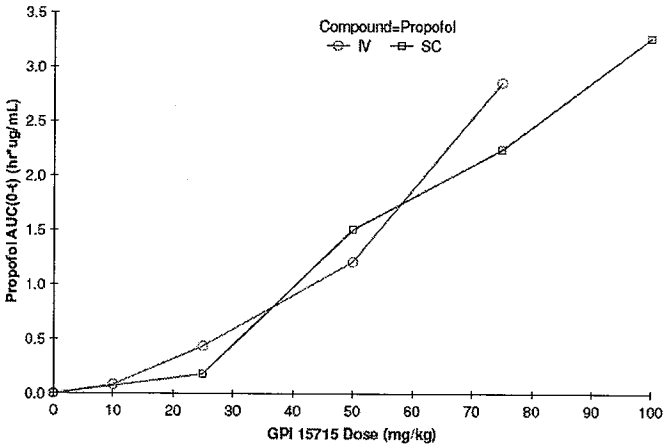


Figure 12: Propofol AUC(0-t) vs. GPI 15715 Dose (mg/kg) Following Administration of a Single Intravenous Injection Dose of 10, 25, 50, and 75 mg/kg GPI 15715 and a Single Subcutaneous Injection Dose of 25, 50, 75, and 100 mg/kg GPI 15715 to Male Rats



Study title: A Pharmacokinetic Study of Single Dose of AQUAVAN® or DIPRIVAN® Administered by Intravenous Bolus Injection, Gastric Bypass and Oral (Gavage), Routes to Male Rats

Study number: Absorp\DMPK-06-102

Study design: Male rats (n= 3) was administered either with GPI 15715 or with propofol at different doses. The route of administration was intravenous, oral, or gastric bypass. Following is the Sponsor's study deign.

Table A: Experimental Design:

Study Group	Route of Administration	Number of Animals	Gender	Test Material	Dose Level (mg/kg)	Dose Concentration (mg/mL)
1	Intravenous Bolus	3	Male	GPI 15715 [a]	5	5.0
	Oral	3	Male	GPI 15715 [a]	20	10.0
		3	Male	GPI 15715 [a]	50	25.0
		3	Male	GPI 15715 [a]	100	50.0
2	Intravenous Bolus	2	Male	GPI 15715 [a]	50	50.0
	Gastric Bypass	3	Male	GPI 15715 [a]	1	0.5
		3	Male	GPI 15715 [a]	3	1.5
		3	Male	GPI 15715 [a]	10	5.0
		3	Male	GPI 15715 [a]	30	15.0
		3	Male	GPI 15715 [a]	100	50.0
3	Intravenous Bolus	3	Male	DIPRIVAN® [b]	3	3.0
	Oral	3	Male	DIPRIVAN® [b]	10	2.0
		3	Male	DIPRIVAN® [b]	25	5.0
		3	Male	DIPRIVAN® [b]	50	10.0
4	Intravenous Bolus	3	Male	DIPRIVAN® [b]	15	5.0
		3[c]	Male	DIPRIVAN® [b]	30	10.0
	Gastric Bypass	3	Male	DIPRIVAN® [b]	1	0.2
		3	Male	DIPRIVAN® [b]	5	1.0
		3	Male	DIPRIVAN® [b]	15	3.0
		3	Male	DIPRIVAN® [b]	30	6.0
		3	Male	DIPRIVAN® [b]	50	10.0

[a] GPI 15715 for Injection (35 mg/mL) diluted with saline

[b] DIPRIVAN® for Injection (10 mg/mL) diluted with 20% lipid as required

[c] Two rats in this treatment died after dose administration

Results and Reviewer's Comments: The Cmax of GPI 15715 and propofol derived from GPI 15715 increased dosages proportionally after the oral, gastric bypass, and IV administration. The T½ ranged between 0.19-0.51 hr following oral, gastric bypass, and IV administration of the test article. The mean terminalT½ of the propofol derived from GPI 15715 after intravenous, oral, and gastric bypass administration ranged between

1.63-2.43 hr, 3.07-4.66 hr, and 2.32-2.85 hr respectively. The oral bioavailability of the propofol derived GPI 15715 after 20, 50, and100 mg/kg of the test article administration were 22, 7, and 70% respectively. The bioavailability of the propofol derived GPI 15715 after 10, 30, and100 mg/kg of the administration of the test article via gastric bypass were 33, 45, and 135% respectively. The mean terminal T½ of propofol after its administration via i.v, oral, and gastric bypass were 1.68-4.6 hr, 2.45-3.77 hr, and 1.84-4.86 hr respectively. The bioavailability of the propofol after its oral administration of 25 and 50 mg/kg of the test article administration were 18 and 20% respectively. The bioavailability of the propofol after 5, 15, 30, and 50 mg/kg of the administration of propofol via gastric bypass were 14, 14, 23, and 38% respectively.

Table 3. Mean (SD) pharmacokinetic parameters of GPI 15715 following GPI 15715 administrations (Group 1 and Group 2)

Study Group	Route	Dose (mg/kg)	N		T_{max}[†] (min)	C_{max} (µg/mL)	$AUC_{(0-t)}$ (µg•hr/mL)	$AUC_{0-\infty}$ (µg•hr/mL)	F[§] (%)	$T_{1/2}$ (hr)	V_d (L/kg)	CL_p (L/hr/kg)
1	IV	5	3	Mean	5.0	16.3	3.15	3.15	ND	0.49	1.02	1.38
				SD	(5.0-5.0)	1.80	0.285	0.281		0.28	0.658	0.125
	Oral	20	3	Mean	5.0	0.228	0.0527	0.0564	0.448	0.19	ND	ND
				SD	(5.0-5.0)	0.0723	0.00809	0.00747		0.02	ND	ND
	Oral	50	3	Mean	5.0	2.41	0.846	0.864	2.74	0.51	ND	ND
				SD	(5.0-15.0)	2.28	0.962	0.960		0.32	ND	ND
	Oral	100	3	Mean	5.0	9.23	2.16	2.18	3.46	0.49	ND	ND
				SD	(5.0-5.0)	4.09	0.993	0.997		0.24	ND	ND
2	IV	50	2	Mean	5.0	74.7	19.1	12.1[#]	ND	0.23[#]	1.44[#]	4.38[#]
				SD	(5.0-5.0)	31.7	10.0	ND[◊]		ND	ND	ND
	GB	1	3	Mean	17.5[‡]	0.0396[‡]	0.0142[‡]	ND[◊]	ND	ND	ND	ND
				SD	(5.0-30.0)	0.0204	0.00439	ND[◊]		ND	ND	ND
	GB	3	3	Mean	22.5[‡]	0.0252[‡]	0.0194[‡]	ND[◊]	ND	ND	ND	ND
				SD	(15.0-30.0)	0.00453	0.00140	ND[◊]		ND	ND	ND
	GB	10	3	Mean	5.0[‡]	0.0291[‡]	0.0121[‡]	ND[◊]	ND	ND	ND	ND
				SD	(5.0-5.0)	ND	ND	ND[◊]		ND	ND	ND
	GB	30	3	Mean	5.0[#]	0.169	0.0440	0.0482	0.811	0.21	ND	ND
				SD	(5.0-5.0)	ND	ND	ND[◊]		ND	ND	ND
	GB	100	3	Mean	5.0	3.74	0.644	0.652	3.29	0.23	ND	ND
				SD	(5.0-5.0)	2.12	0.378	0.386		0.10	ND	ND

[†]Median (range)
ND: not determined
[§]Ratio of mean of $AUC_{(0-\infty)}$ of test to reference treatment. For oral and gastric bypass, reference treatments were 5 mg/kg and 50 mg/kg intravenous dose of GPI-15715, respectively
[◊]Not determined as terminal elimination rate constant could not be calculated.
[#]N = 1
[‡] N = 2

Table 4. Mean (SD) pharmacokinetic parameters of Propofol following GPI 15715 administrations (Group 1 and Group 2)

Study Group	Route	Dose (mg/kg)	N		T_{max}† (min)	C_{max} (µg/mL)	$AUC_{(0-t)}$ (µg•hr/mL)	$AUC_{(0-\infty)}$ (µg•hr/mL)	F‡ (%)	$T_{1/2}$ (hr)
1	IV	5	3	Mean	5.0	0.286	0.118	0.139	ND	1.63
				SD	(5.0-5.0)	0.0400	0.0210	0.0330		0.87
	Oral	20	3	Mean	5.0	0.0426	0.0608	0.126	22.7	4.66
				SD	(5.0-5.0)	0.000922	0.0429	0.122		4.13
	Oral	50	3	Mean	5.0	0.133	0.254	0.103	7.41	3.07
				SD	(5.0-15.0)	0.0899	0.156	ND§		ND
	Oral	100	3	Mean	5.0	0.526	1.21	1.96	70.5	4.13
				SD	(5.0-5.0)	0.0829	0.203	0.606		1.12
2	IV	50	2	Mean	5.0	2.48	1.32	1.46	ND	2.43
				SD	(5.0-5.0)	0.297	0.392	0.404		0.22
	GB	1	3	Mean	ND	ND	ND	ND§	ND	ND
				SD	ND	ND	ND	ND§		ND
	GB	3	3	Mean	ND	ND	ND	ND§	ND	ND
				SD	ND	ND	ND	ND§		ND
	GB	10	3	Mean	5.0	0.200	0.0598	0.0973‡	33.3	2.49‡
				SD	(5.0-15.0)	0.108	0.0249	0.0218		2.35
	GB	30	3	Mean	5.0	1.27	0.353	0.398	45.4	2.85
				SD	(5.0-5.0)	0.871	0.143	0.135		0.87
	GB	100	3	Mean	5.0	5.84	3.57	3.95	135	2.32
				SD	(5.0-5.0)	2.29	0.568	0.477		0.73

†Median (range)
ND: not determined
§Not determined as terminal elimination rate constant cpuld not be calculated
‡Ratio of mean of $AUC_{(0-\infty)}$ of test to reference treatment. For oral and gastric bypass, reference treatments were 5 mg/kg and 50 mg/kg intravenous dose of GPI 15715, respectively
‡ N = 2

Study title: Assessment of the Pharmacokinetics of AQUAVAN (GPI 15715) Following Intravenous, Oral Gavage, Intraduodenal Port, Intrarectal, and Subcutaneous Administration to Male Non-naïve Beagle Dogs

Study number: Absorp\04-guil.p01r1 & 4guilp8

Study design: In this study the pharmacokinetics of GPI 15715 was determined in dog after its administration through different routes such as IV, oral, subcutaneous, intraduodenal, and intrarectal. Following is the Sponsor's study design table.

Table 11
Study Design

Date of Dose	Dog	Weight (kg)	Volume Dosed (mL)	Route of Administration	Dosing Volume (mL/kg)	Dosing Conc. (mg/mL)	Dose (mg/kg)
April 8, 2004	46	9.5	2 suppositories*	Intrarectal (IR)	N/A	160 mg	16.8*
	47	10.2	1 suppository		N/A	160 mg	15.7
	48	8.7	1 suppository		N/A	160 mg	18.4
April 15, 2004	46	9.9	4.5	Intraduodenal Port (IDP)	0.457	35	16
	47	10.8	4.9		0.457	35	16
	48	9.0	4.1		0.457	35	16
April 19, 2004	46	10.2	4.7	Intravenous (IV)	0.457	35	16
	47	10.8	4.9		0.457	35	16
	48	9.1	4.2		0.457	35	16
April 23, 2004	46	10.0	4.6	Oral Gavage (OG)	0.457	35	16
	47	10.8	4.9		0.457	35	16
	48	9.0	4.1		0.457	35	16
April 28, 2004	46	10.3	0.8	Subcutaneous (SC)	0.08	200	16
	47	10.9	0.9		0.08	200	16
	48	9.0	0.7		0.08	200	16

*Dog 46 expelled the first suppository at ~2 minutes post-dose. A second suppository was then administered.
N/A: Not Applicable

Results and Reviewer's Comments: As shown in the Sponsor's table, the Cmax (3.12 µg/mL) of the GPI 15715 derived propofol was highest after intravenous administration of the test article. Similarly the exposure as described by AUC_{last} (1585 ng.h/mL) was also highest after intravenous administration of the test article.

Table 4
Average Pharmacokinetic Parameters (N=3) of Propofol After Administration of AQUAVAN® to Male Beagle Dogs

Route of Administration	AUC_{Last}[1] (hr·ng/mL)	C_{max} (µg/mL)	T_{max} (hr)
Intrarectal	220.5 ± 186.6	0.15 ± 0.07	0.50 ± 0.43
Intraduodenal	46.5 ± 17.3	0.08 ± 0.02	0.19 ± 0.10
Intravenous	1585 ± 87.7	3.12 ± 0.80	0.03 ± 0
Oral Gavage	99.5[2]	0.04 ± 0.03	1.0 ± 0.5
Subcutaneous	1038 ± 319.8	0.31 ± 0.06	1.3 ± 0.29

[1]AUC values are not normalized to dose
[2]Average of N=2

Study title: Preliminary Pharmacokinetics of GPI 15715 in the Rat

Study number: Absorp\DM-00-011

Study design: In a preliminary study, to evaluate the pharmacokinetics of propofol derived from GPI 15715, the Sponsor administered 10 mg/kg of the test article via jugular vein in male rats (n=5).

Results and Reviewer's Comments: Based on a noncompartmental model the Cmax of propofol after the IV administration of GPI 15715 reached at 5 mins suggesting rapid degradation. Based on the concentrations of propofol and GPI 15751, the bioavailability of propofol (F) was 0.251. GPI 15715 was observed to be distributed in rat biexponentially when examined in a noncompartmental analysis. In this analysis, the half life of propofol in the two phases was 2.3 and 19.4 mins respectively and the apparent volume of GPI 15715 distributions in the main compartment was 27 mL for propofol. The analyses also suggest that the mean residual time of GPI 15715 was higher (21 mins) compare to propofol derived from GPI 15715 (9 mins). The two compartmental analyses showed that the half life of propofol for the two phases was 2.9 and 133 mins respectively and the apparent volume of distribution in the main compartment was 60 mL for propofol.

Study title: Study DM-00-012: Preliminary Pharmacokinetics of GPI 15715 in Dogs

Study number: Absorp\DM-00-012Absorp

Study design: In a preliminary study, to evaluate the pharmacokinetics of propofol derived from GPI 15751, the Sponsor administered 7 mg/kg of propofol or GPI 15715 via cephalic vein in dogs (n=3) in a cross over study.

Results and Reviewer's Comments: Based on a non compartmental model the Cmax of propofol after the IV administration of GPI 15715 reached at 6 mins. Propofol was metabolized from GPI 15715 rapidly ($T_{1/2}$= 7 mins) after the IV administration of GPI 15715 suggesting rapid degradation. The peak concentration of propofol after its direct administration was much higher 50-60 μM compared than that of GPI 15715 administrations (7-8 μM).

Based on the concentrations of propofol and GPI 15715, the bioavailability of propofol (F) was 0.843. The apparent volume distribution and system clearance of propofol after its direct administration were 8.94 L and 0.929 L/min respectively. The apparent volume distribution and system clearance of propofol after the administration of GPI 15715 were 7.21 L and 0.929 /min respectively.

2.6.4.4 Distribution

Study title: *In Vitro* Plasma Protein Binding, Protein Binding Interaction, and Blood-to-Plasma Partitioning of [^{14}C] GPI 15715 in Mouse, Rat, Rabbit, Dog, Monkey, and Human

Study number: Distrib/6778-145

Study design: The in vitro protein binding and blood to plasma partitioning of the ^{14}C-GPI 15715 were determined by ultra filtration at different concentrations in monkey, dog, rabbit, rat, and mice. In addition, the binding of GPI 15715 was determined in human serum albumin (HSA) as well as α1 acid glycoprotein (AAG). The interaction of propofol with GPI 15715 was also determined. The studies were conducted under GLP in the [redacted] b(4)

Results and Reviewer's Comments: GPI 15715 was found to be highly protein bound. The binding in mouse, rat, rabbit, dog, monkey, and human were 93, 97, 91, 95, 96, and 97 % respectively between 0.5-100 µg/mL concentrations. In concentrations higher than 100 µg/mL, the protein binding in all of the above mentioned species studied was significantly lower indicating saturation of the protein binding sites. GPI 15715 was highly bound to HSA (93%) but minimally bound to AAG (<10%). The potential for GPI 15715 and propofol interaction was found to be minimal up to a concentration of 200 µg/mL.

Study title: 6778-152 (PK-SMP-15715-001): Tissue Distribution of ^{14}C-GPI 15715 After Administration of an Intravenous Dose to Male Rats

Study number: Distrib/6778-152

Study design: The tissue distribution of the 14C-GPI 15715 was examined after an IV administration of 20 mg/kg in male Long Evans rat under GLP conditions in the [redacted] b(4) Three animals /time point was sacrificed at 0.083, 1, 3, 8, 24, 72, and 120 hrs post dose.

Results and Reviewer's Comments: The maximum concentration of the radioactivity in blood and plasma was 45 and 86 µg respectively at 0.083 mins (Tmax). These concentrations were observed to be declined with time and appeared to be neglible at 120 hr post dosing. The elimination T½ was 80 and 70 hrs in blood and plasma respectively. Note that at earlier time point the concentration of the radioactive GPI 15715 was higher in plasma, but after 24 hrs the concentration of the test article was higher in blood indicating that the test article was distributed preferentially in the cellular component of the blood (Sponsor's table 2). The tissues with the highest concentration of the radioactivity at 0.083 mins were adrenal gland, liver, kidney, bone marrow, salivary gland, thyroid, with and lungs with concentration of 81.2,72.2, 47.5, 45.4, 34.2, 24.9, and

19.8 µg respectively. The Cmax in the small intestine (@ 3hrs), large intestine (@ 8hrs), and fat (@ 1hrs) were 317, 214, and 44.5 µg respectively. The delayed Cmax in the intestine suggest biliary excretion as a major pathway for elimination of the test article. The Cmax in the brain, eye, and skin was observed at 0.083 mins and was found to be 9.95, 2.91, and 12.0 µg respectively (Sponsor's table 4). The tissue to plasma ratios of the GI tract tissues are in general higher than the rest of the tissue studied. 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 120 hrs post dosing, 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.

Table 3
Pharmacokinetic parameters for total radioactivity in blood and plasma collected from male rats following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg)

Matrix	C_{max} (µg equiv/g)	T_{max} (hours)	$t_{½}$ (hour)	AUC_{0-t} (µg equiv · hour/g)	$AUC_{0-\infty}$ (µg equiv · hour/g)
Blood	45.0	0.083	80.1	156.6	218.5
Plasma	86.0	0.083	70.1	148.8	159.6

equiv Equivalents.

Table 4
Mean concentrations of radioactivity in blood, plasma, and tissues at specified times following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg) to male rats

	µg Equivalents ^{14}C-GPI 15715/g					
	0.083 Hours		1 Hours		3 Hours	
Matrix	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	81.2	15.6	8.29	1.30	2.58	1.15
Bladder (urinary)	12.7	1.9	5.25	0.07	2.62	0.85
Blood	45.0	2.7	7.71	0.12	3.49	0.33
Bone (both femurs)	7.39	0.38	2.56	0.28	1.09	0.14
Bone marrow (both)	45.4	28.7	2.98	0.01	1.44	0.12
Brain	9.95	1.14	2.06	0.14	0.571	0.087
Eyes (both)	2.91	0.15	1.57	0.01	0.594	0.032
Fat (reproductive)	10.6	3.2	44.5	1.8	27.5	6.0
Heart	19.1	0.7	4.15	0.14	1.54	0.06
Kidneys	47.5	7.5	20.3	2.0	9.08	0.49
Large Intestine	9.04	1.48	5.27	0.75	136	97
Liver	72.2	7.4	36.0	2.0	16.9	1.7
Lungs	19.8	1.8	5.96	0.66	2.48	0.14
Lymph Nodes (mesenteric)	14.1	1.4	11.5	1.6	7.71	4.77
Muscle (thigh)	8.46	0.11	2.65	0.37	1.29	0.29
Pancreas	15.3	1.3	6.98	1.10	4.50	2.96
Plasma	86.0	2.5	12.2	0.3	5.00	0.46
Prostate	14.6	1.5	8.46	1.70	3.63	0.23
Salivary glands	34.2	0.8	5.28	0.41	1.98	0.21
Skin (pigmented)	12.0	0.3	6.65	0.42	2.25	0.63
Small Intestine	29.0	4.6	354	24	376	99
Spleen	7.81	0.93	2.35	0.11	1.12	0.05
Stomach	14.1	3.3	12.1	4.2	9.78	6.74
Testes	5.59	0.61	2.15	0.08	0.912	0.123
Thymus	8.56	1.80	2.55	0.38	0.893	0.078
Thyroid	24.9	3.5	4.53	1.00	1.42	0.25

SD Standard deviation.

Table 4 (continued)

Mean concentrations of radioactivity in blood, plasma, and tissues at specified times following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg) to male rats

	µg Equivalents ^{14}C-GPI 15715/g					
	8 Hours		24 Hours		72 Hours	
Matrix	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	1.07	0.11	0.456	0.056	0.0940	0.163
Bladder (urinary)	1.29	0.56	0.871	0.410	0.00	0.00
Blood	2.37	0.44	1.23	0.12	0.652	0.015
Bone (both femurs)	0.491	0.033	0.338	0.023	0.224	0.060
Bone marrow (both)	1.01	0.14	0.623	0.121	0.217	0.188
Brain	0.266	0.040	0.159	0.008	0.111	0.022
Eyes (both)	0.330	0.043	0.210	0.022	0.0864	0.0751
Fat (reproductive)	15.4	3.8	1.68	0.29	0.127	0.022
Heart	0.891	0.106	0.426	0.076	0.249	0.033
Kidneys	4.83	1.11	2.31	0.46	0.564	0.008
Large Intestine	214	87	13.3	6.0	0.563	0.285
Liver	12.4	1.7	2.54	1.10	0.550	0.043
Lungs	1.59	0.16	0.808	0.181	0.432	0.052
Lymph Nodes (mesenteric)	2.02	1.06	0.441	0.110	0.0950	0.0839
Muscle (thigh)	0.734	0.036	0.491	0.042	0.350	0.031
Pancreas	1.88	0.46	0.452	0.089	0.136	0.017
Plasma	2.77	0.72	0.822	0.178	0.172	0.005
Prostate	2.14	1.11	0.393	0.348	0.0453	0.0784
Salivary glands	0.853	0.086	0.315	0.041	0.140	0.025
Skin (pigmented)	0.891	0.230	0.384	0.050	0.224	0.040
Small Intestine	133	37	13.5	5.5	0.518	0.263
Spleen	0.778	0.026	0.445	0.065	0.00	0.00
Stomach	1.19	0.64	0.278	0.250	0.0276	0.0478
Testes	0.502	0.106	0.228	0.013	0.110	0.005
Thymus	0.572	0.148	0.347	0.035	0.207	0.018
Thyroid	0.266	0.460	0.00	0.00	0.00	0.00

SD Standard deviation.

Table 4 (continued)

Mean concentrations of radioactivity in blood, plasma, and tissues at specified ti following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg) to m rats

µg Equivalents ^{14}C-GPI 15715/g		
	120 Hours	
Matrix	Mean	SD
Adrenal glands	0.00	0.00
Bladder (urinary)	0.00	0.00
Blood	0.536	0.009
Bone (both femurs)	0.101	0.088
Bone marrow (both)	0.00	0.00
Brain	0.0931	0.0104
Eyes (both)	0.00	0.00
Fat (reproductive)	0.0974	0.0091
Heart	0.00	0.00
Kidneys	0.426	0.010
Large Intestine	0.192	0.028
Liver	0.358	0.036
Lungs	0.266	0.021
Lymph Nodes (mesenteric)	0.00	0.00
Muscle (thigh)	0.291	0.010
Pancreas	0.00	0.00
Plasma	0.107	0.009
Prostate	0.00	0.00
Salivary glands	0.0782	0.0684
Skin (pigmented)	0.176	0.015
Small Intestine	0.150	0.030
Spleen	0.00	0.00
Stomach	0.0274	0.0475
Testes	0.0770	0.0017
Thymus	0.0757	0.0660
Thyroid	0.00	0.00

SD Standard deviation.

Table 5
Mean tissue:plasma concentration ratios at specified times following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg) to male rats

Tissue:Plasma Concentration Ratios						
	0.083 Hours		1 Hour		3 Hours	
Matrix	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	0.948	0.211	0.676	0.088	0.515	0.218
Bladder (urinary)	0.147	0.023	0.429	0.007	0.520	0.135
Blood	0.523	0.017	0.630	0.008	0.698	0.009
Bone (both femurs)	0.0859	0.0049	0.209	0.019	0.218	0.016
Bone marrow (both)	0.527	0.327	0.243	0.008	0.289	0.011
Brain	0.116	0.014	0.169	0.015	0.114	0.007
Eyes (both)	0.0338	0.0025	0.128	0.004	0.119	0.005
Fat (reproductive)	0.124	0.039	3.64	0.25	5.44	0.75
Heart	0.222	0.011	0.339	0.012	0.309	0.037
Kidneys	0.553	0.094	1.66	0.14	1.83	0.28
Large Intestine	0.105	0.016	0.430	0.062	28.3	21.3
Liver	0.841	0.092	2.94	0.15	3.38	0.27
Lungs	0.231	0.026	0.486	0.050	0.497	0.025
Lymph Nodes (mesenteric)	0.165	0.020	0.936	0.116	1.51	0.83
Muscle (thigh)	0.0984	0.0018	0.216	0.027	0.263	0.085
Pancreas	0.177	0.011	0.569	0.079	0.888	0.555
Prostate	0.170	0.015	0.689	0.125	0.727	0.044
Salivary glands	0.397	0.006	0.431	0.027	0.396	0.043
Skin (pigmented)	0.139	0.006	0.542	0.021	0.447	0.102
Small Intestine	0.338	0.062	29.0	2.7	75.0	16.8
Spleen	0.0909	0.0129	0.192	0.011	0.226	0.021
Stomach	0.165	0.043	0.987	0.333	2.05	1.59
Testes	0.0650	0.0081	0.176	0.005	0.182	0.009
Thymus	0.0999	0.0235	0.208	0.026	0.179	0.016
Thyroid	0.289	0.046	0.369	0.074	0.282	0.026

SD Standard deviation.

Table 5 (continued)
Mean tissue:plasma concentration ratios at specified times following a single intravenous administration of ^{14}C-GPI 15715 (20 mg/kg) to male rats

	Tissue:Plasma Concentration Ratios					
	8 Hours		24 Hours		72 Hours	
Matrix	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	0.409	0.128	0.580	0.186	1.70	NA
Bladder (urinary)	0.479	0.226	1.02	0.32	NA	NA
Blood	0.865	0.059	1.52	0.21	3.80	0.19
Bone (both femurs)	0.184	0.037	0.424	0.096	1.31	0.39
Bone marrow (both)	0.372	0.051	0.783	0.236	1.86	NA
Brain	0.0980	0.0135	0.202	0.057	0.647	0.141
Eyes (both)	0.122	0.018	0.261	0.032	0.743	NA
Fat (reproductive)	5.67	1.28	2.06	0.13	0.740	0.133
Heart	0.330	0.043	0.522	0.025	1.45	0.19
Kidneys	1.76	0.07	2.81	0.11	3.29	0.09
Large Intestine	85.5	52.5	15.6	4.8	3.25	1.59
Liver	4.58	0.59	3.00	0.70	3.20	0.16
Lungs	0.589	0.098	1.02	0.33	2.52	0.31
Lymph Nodes (mesenteric)	0.737	0.406	0.534	0.031	0.816	NA
Muscle (thigh)	0.275	0.058	0.613	0.111	2.04	0.24
Pancreas	0.731	0.305	0.559	0.100	0.792	0.082
Prostate	0.807	0.456	0.641	NA	0.785	NA
Salivary glands	0.318	0.056	0.390	0.054	0.814	0.144
Skin (pigmented)	0.324	0.052	0.492	0.179	1.31	0.24
Small Intestine	52.2	25.9	15.9	3.9	3.00	1.48
Spleen	0.294	0.078	0.548	0.048	NA	NA
Stomach	0.434	0.231	0.395	0.433	0.478	NA
Testes	0.183	0.011	0.287	0.068	0.644	0.044
Thymus	0.215	0.070	0.432	0.069	1.21	0.13
Thyroid	0.310	NA	NA	NA	NA	NA

SD Standard deviation.

2.6.4.5 Metabolism

Study title: A Preliminary Study of the Metabolic Stability in Mouse, Rat, Dog, and Human Microsomes

Study number: Metab\dm-00-021

Study design: The metabolic stability of GPI 15715 was determined over a 2-hr time period in an in vitro assay from the microsomes of mouse, rat, dog, and human at 100 μM concentration.

Results and Reviewer's Comments: The metabolic stability of GPI 15715 in mouse, rat, dog and human in the presence and the absence of the NADPH cofactor were

observed to be approximately 73, 52, 19, and 66% respectively indicating that GPI 15715 underwent metabolism. Because NADPH did not affect the metabolism it appears that minimal CYP 450 based metabolism occurred at this time period.

Figure 1. Metabolic Stability of GPI 15715 in Mouse Microsomes

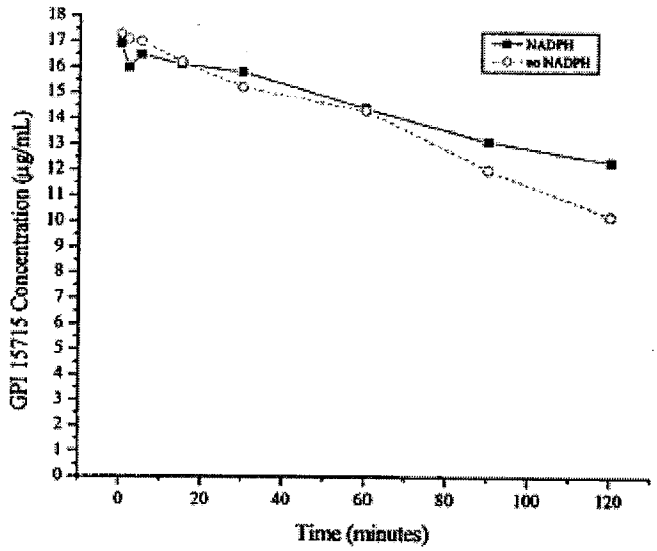


Figure 2. Metabolic Stability of GPI 15715 in Rat Microsomes

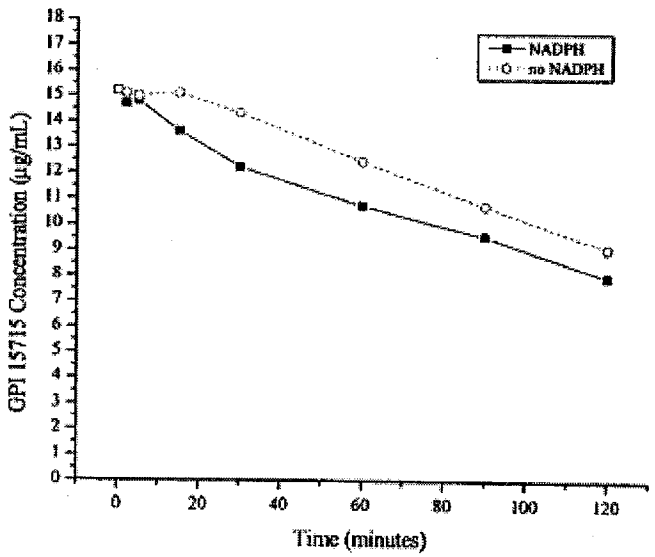


Figure 3. Metabolic Stability of GPI 15715 in Dog microsomes

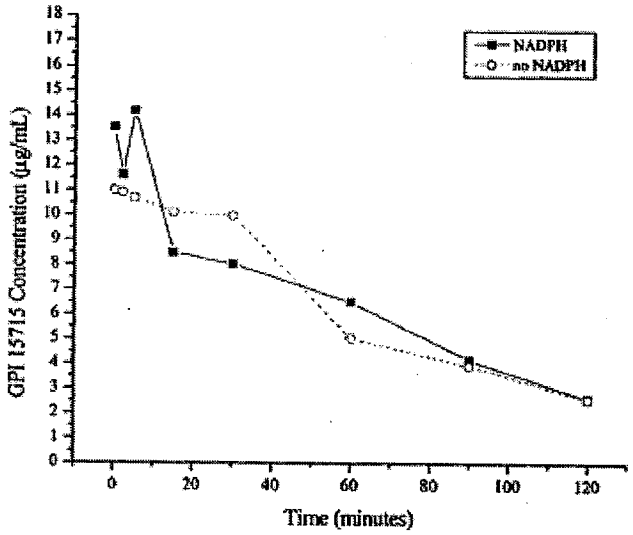


Figure 4. Metabolic Stability of GPI 15715 in Human Microsomes