Bacterial Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and WP2 *uvr*A.

Doses used in definitive study: Concentrations of 33.3, 100, 333, 1000, and 5000 µg/plate for GPI 15715 were tested in both the presence and absence of metabolic activation.

Basis of dose selection: Doses used in initial cytotoxicity-mutation assay ranged from 6.67 to 5000 µg/plate for GPI 15715, one plate per dose, both in presence and absence of the metabolic activation system. The test article was soluble and clear in water at the highest concentrations tested. Neither precipitate nor appreciable toxicity was observed. No positive mutagenic responses were observed in any tester strains either with or without S9 activation. The definitive study therefore used the maximum recommended concentrations of test article as the basis for dose selection.

Negative controls: Water

Positive controls: Refer to the following table.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	Rat	Benzo[a]pyrene	2.5
TA100, 1535, 1537		2-aminoanthracene	2.5
WP2*uvr*A			25.0
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		Sodium azide	2.0
TA1537		ICR-191	2.0
WP2 *uvr*A		4-nitroquinoline-N-oxide	1.0

Comments: Controls are acceptable according to current standards.

Incubation and sampling times: 48 to 72 hours at 37°C,

Results:

Study validity: The study appears to be valid for the following reasons: 1) the appropriate controls were used, 2) the appropriate strains were tested, 3) the positive control substances produced reliable positive results, 4) the highest concentration of GPI 15715 tested reached the maximum recommended concentration that is 5,000 µg/plate, and 5) there was no evidence for a dose dependent increase in revertants following drug treatment.

Study outcome: The test article did not produce any increases in the number of revertants in any tester stain under the conditions tested (refer to the Sponsor's table 4). **This is in concurrence with the Sponsor's conclusions.**

TABLE 4 : MUTAGENICITY ASSAY RESULTS - SUMMARY

TEST ARTICLE ID: GPI-15715

EXPERIMENT ID: 21706-B1

DATE PLATED: 31-Aug-00 VEHICLE: Water

DATE COUNTED: 05-Sep-00 PLATING ALIQUOT: 50 µL

	DOSE/PLATE	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACK-GROUND LAWN*
		TA98		TA100		TA1535		TA1537		WP2*uvrA*		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: RAT LIVER												
VEHICLE CONTROL		26	3	108	6	12	5	7	2	23	5	1
TEST ARTICLE	33.3 µg	31	7	91	15	16	2	8	1	20	5	1
	100 µg	30	1	102	18	12	4	6	2	35	5	1
	333 µg	32	3	113	4	13	3	4	2	43	1	1
	1000 µg	30	3	115	23	14	2	7	3	14	4	1
	3330 µg	37	12	132	8	9	3	10	2	17	4	1
	5000 µg	27	6	104	13	14	1	5	2	20	4	1
POSITIVE CONTROL**		218	7	865	64	161	22	181	10	150	8	1
MICROSOMES: NONE												
VEHICLE CONTROL		13	2	85	10	12	4	7	3	15	6	1
TEST ARTICLE	33.3 µg	14	6	87	10	15	9	7	1	12	5	1
	100 µg	16	3	98	7	14	1	7	0	18	5	1
	333 µg	15	4	96	14	12	2	6	2	22	6	1
	1000 µg	17	4	110	17	16	3	6	1	18	2	1
	3330 µg	15	4	98	2	10	3	5	1	17	4	1
	5000 µg	14	3	100	12	12	2	6	4	22	2	1
POSITIVE CONTROL***		159	13	616	9	595	56	542	51	224	17	1

** TA98 benzo[a]pyrene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2*uvrA* 2-aminoanthracene 25.0 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2*uvrA* 4-nitroquinoline-N-oxide 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced
4 = extremely reduced 5 = absent 6 = obscured by precipitate
sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

Study title: L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with GPI 15715

Key findings

- GPI 15715 was tested in the L5178Y TK$^{+/-}$ forward mutation assay at concentrations of 1000-3400 µg/mL in the absence of metabolic activation and 5-500 µg/mL in the presence of metabolic activation.
- There was no evidence for cytotoxicity or mutation at any concentration in the absence of metabolic activation. However, in the presence of metabolic activation there was an increase in the mutant frequency. The test article was therefore evaluated as positive in the presence of the metabolic activation system.

Study number: 3000-15715-tox-00-11g

Volume # and page #: eCTD submission; Page 1-221
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: August 16, 2000
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715; Lot #21708-21-3; Purity: 84.8%

Methods: The principle of the assay lies in the fact that if the thymidine analog 5-triflurothymidine (TFT) is included in the growth medium, the analog will be phosphorylated via the thymidine kinase (TK) pathway and will cause cell death by inhibiting DNA synthesis. Cells which are heterozygous at the TK ($TK^{+/-}$) locus may undergo a single step forward mutation to the $TK^{-/-}$ in which no TK activity remains. Such mutants are viable in culture because *de novo* DNA synthesis does not require exogenous thymidine. The survival of the colonies, therefore, depends either on the spontaneous mutation or the forward mutation at the TK locus induced by the test article.

The assay was conducted in two phases to evaluate the colony growth of $L5178Y^{+/-}$ (TK) mouse lymphoma cells in the presence of (TFT). The first phase was the dose range finding assay to select doses for the definitive assay. The cytotoxicity end point for this phase of the study was relative suspension growth (RSG) determined by the total cell growth with or without treatment in the presence and absence of the metabolic activation system. In the second phase, the cell cultures were exposed to the test article for 4 hrs in the presence and absence of the metabolic activation system. The cells were then cultured in suspension for a 48 hr expression period. After that the cells were plated in soft agar medium (with or without TFT) for 12 days. Cytotoxicity at this phase was determined by relative total growth (RTG) defined by the treatment related growth not only at the suspension culture phase but also at the cloning phase. The mutant frequency was determined by counting the number of TFT resistant colony. Both large and small colonies were quantified for all cultures. The large colonies presumably arise from point mutations and the small colonies from the chromosomal changes.

Cell line: L5178Y mouse lymphoma cells, heterozygous at the TK locus was used for this assay.

Doses used in definitive study: In the confirmatory assay without metabolic activation, eleven treatments at 62.5, 125, 250, 500, 1000, 1500, 2000, 2500, 3000, and 3400 µg/mL of GPI 15715 were initiated; treatments at and below 750 µg/mL were terminated because there were sufficient other doses available for analysis. The remaining eight doses were used for the mutant analysis. In the confirmatory assay with metabolic activation, eleven treatments at 62.5, 125, 250, 500, 1000, 1500, 2000, 2500, 3000, and 3400 µg/mL of GPI 15715 were initiated; treatments at and above 1000 µg/mL were terminated because of excessive cytotoxicity. The remaining seven concentrations were used for the mutant analysis.

Basis of dose selection: Concentrations used in initial cytotoxicity-mutation assay ranged from 5 to 3400 µg/mL for GPI 15715, one plate per concentration, both in presence and

absence of the metabolic activation system. The test article was soluble and clear in water at the highest concentrations tested. No precipitate was observed. The dose in the confirmatory assay with metabolic activation was based on the cytotoxicity.

Negative controls: The vehicle of choice for the test article was water.

Positive controls: The positive control articles used in the assay in the presence and absence of the metabolic activation system were methylcholanthrene and methyl methane sulfonate respectively. Controls are acceptable according to the current standards.

Incubation and sampling times: The cells were treated for 4 hrs in the presence and absence of the metabolic activation system at 37°C. The cells were then cultured in suspension for a 48 hrs expression period. After that the cells were plated in soft agar medium (with or without TFT) for 12 days.

Results:

Study validity: The study appeared to be valid for the following reasons: 1) the appropriate controls were used, 2) vehicle control cultures exhibited a mean cloning efficiency of 50% or greater, 3) vehicle control cultures exhibited a mean mutant frequency less that $150x10^{-6}$, 4) the positive control substances produced reliable positive results, 5) the dose selection was based on approximately 80% RTG, 6) the ability to recover small colonies was demonstrated by the sizing of the small colonies.

Study outcome: The test article GPI 15715, was concluded to be positive (refer to table 10) under metabolic activation for inducing forward mutation at the TK locus in L5178Y mouse lymphoma cells and negative (refer to table 16) under non activation conditions used in this study. This is in concurrence with the Sponsor's conclusions.

TABLE 6: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE:GPI-15715
B. GENETICS ASSAY NO.: 21706-0-431 ICH
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 μg/mL
E. TREATMENT DATE: 09/19/2000

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c] (%)	Mutant Frequency ($x10^{-6}$)[d] Total	Small	Large
Vehicle Control[e]										
	10%	130.7	100.7	106.3	74.0	84.6	89.8	77.2	49.0	28.2
	10%	78.2	83.5	111.6	86.7	99.1	110.6	55.3	31.9	23.5
	10%	91.1	115.8	82.1	101.8	116.3	95.5	58.1	33.1	25.0
MMS[f] (μg/mL)										
	6.5	76.4	34.9	28.9	48.4	55.3	16.0	409.0	231.6	177.4
	6.5	112.3	36.1	29.6	40.9	46.7	13.8	445.3	262.2	183.1
Test Article(μg/mL)										
	1000	66.3	60.6	88.2	68.6	78.3	69.1	56.2	28.1	28.1
	1500	46.0	63.1	61.4	86.0	98.3	60.4	58.4	24.9	33.4
	2000	43.3	38.8	41.7	66.4	75.8	31.6	93.7	39.5	54.2
	2500	55.2	32.7	38.7	79.6	91.0	35.2	73.5	37.0	36.5
	2800	50.6	32.6	36.2	66.7	76.2	27.6	91.0	50.7	40.3
	3000	36.8	24.9	25.9	78.7	90.0	23.3	112.2	70.7	41.6
	3200	42.3	24.7	35.1	63.5	72.5	25.5	108.3	74.5	33.8
	3400	54.3	29.9	30.5	86.4	98.7	30.0	95.2	55.6	39.6

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = Water
[f]Positive Control: MMS = Methyl methanesulfonate
Colony Counts increased by 9.099% to compensate for area of dish not scanned

TABLE 10: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: GPI-15715
B. GENETICS ASSAY NO.: 21706-0-431 ICH
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 09/19/2000

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c] (%)	Mutant Frequency ($x10^{-6}$)[d] Total	Mutant Frequency ($x10^{-6}$)[d] Small	Mutant Frequency ($x10^{-6}$)[d] Large
Vehicle Control[e]									
	10%	99.8	102.9	88.4	93.5	96.2	94.2	49.0	45.3
	10%	107.0	95.7	89.5	94.7	90.6	54.5	25.6	28.9
	10%	93.2	101.4	105.6	111.8	113.3	49.2	24.1	25.1
MCA[f] (µg/mL)									
	2	75.0	56.2	114.6	121.2	68.1	165.1	67.6	97.5
	4	76.5	59.1	74.9	79.3	46.9	292.7	173.8	118.9
Test Article(µg/mL)									
	5.00	94.7	106.8	108.0	114.3	122.1	72.1	45.8	26.3
	10.0	94.7	95.3	102.4	108.3	103.3	59.7	30.2	29.5
	25.0	95.4	99.9	104.7	110.8	110.7	53.8	33.3	20.5
	50.0	76.5	66.5	112.2	118.7	79.0	79.1	37.3	41.8
	100	78.6	74.8	106.7	113.0	84.5	93.7	56.9	36.8
	200	60.4	46.3	105.1	111.2	51.5	172.0	118.3	53.6
	500	29.9	14.7	88.6	93.7	13.8	312.1	211.5	100.6

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = Water
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

Study title: L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with GPI 15715 (AQUAVAN®): Formaldehyde Effects

Key findings

- GPI 15715 was tested in the L5178Y TK$^{+/-}$ forward mutation assay at concentrations of 100-3400 µg/mL in the presence of metabolic activation.
- The test article was concluded to be positive for inducing forward mutation at the TK locus in L5178Y mouse lymphoma cells in the absence of formaldehyde dehydrogenase (FDH). The test article, however, was scored negative with metabolic activation in the presence of FDH.

Study number: 3000-15715-tox-00-22g
Volume # and page #: eCTD submission; Page 1-114

b(4)

Conducting laboratory and location: [redacted]
Date of study initiation: June 01, 2005
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715; Lot #21708-21-3; Purity: 84.8%

Methods: The current assay was conducted to confirm that the mutagenic activity of the test article which was observed in the study #3000-15715-00-11g was due to the formation of formaldehyde in the presence of the metabolic activation system. The study was performed with metabolic activation in the absence and presence of an enzyme (formaldehyde dehydrogenase (FDH)/NAD+) system. The first phase of the study confirmed the mutagenicity of formaldehyde, demonstrated the elimination by formaldehyde dehydrogenase/NAD+ and provided dose levels for the formaldehyde control in the mutagenicity assays. In these tests, the mutagenicity of formaldehyde was evaluated at concentrations from 0.75 to 6.00 μg/mL (25.0 to 200 μM) with and without formaldehyde dehydrogenase/NAD+. In the presence of metabolic activation and absence of FDH a 7-fold increase in the mutant frequency was observed. Based on these results the confirmatory mouse lymphoma assay was initiated. Dosing was adjusted to compensate for test article activity.

Cell line: L5178Y mouse lymphoma cells, heterozygous at the TK locus was used for this assay.

Doses used in definitive study: In the confirmatory assay with metabolic activation, for treatments at 100, 500, 2500, and 3400 μg/mL of GPI 15715 were initiated; all of the four treatment groups were used for the mutant analysis.

Basis of dose selection: Concentrations used in initial cytotoxicity-mutation assay ranged from 5 to 3400 μg/mL for GPI 15715, one plate per concentration, both in presence and absence of the metabolic activation system. The test article was soluble and clear in water at the highest concentrations tested. No precipitate was observed. The concentration in the confirmatory assay with metabolic activation was based on the cytotoxicity.

Negative controls: The vehicle of choice for the test article was water.

Positive controls: The positive control articles used in the assay in the presence of metabolic activation system was methylcholanthrene. The selection of controls is acceptable according to the current standards.

Incubation and sampling times: The cells were treated for 4 hrs in the presence and absence of the metabolic activation system at 37°C. The cells were then cultured in suspension for a 48 hr expression period. After that the cells were plated in soft agar medium (with or without TFT) for 12 days.

Results:

Study validity: The study appeared to be valid for the following reasons: 1) the appropriate controls were used, 2) vehicle control cultures exhibited a mean cloning efficiency of 50% or greater, 3) vehicle control cultures exhibited a mean mutant frequency less that $150x10^{-6}$, 4) the positive control substances produced reliable positive results, 5) the dose selection was based on approximately 80 % RTG, and 6) the ability to recover small colonies was demonstrated by the sizing of the small colonies.

Study outcome: The test article GPI 15715, was concluded to be positive (refer to table 7 and 8) with metabolic activation for inducing forward mutation at the TK locus in L5178Y mouse lymphoma cells in the absence of FDH. The test article, however, was scored negative (refer to table 10) with metabolic activation in the presence of FDH. This is in concurrence with **the Sponsor's conclusions.**

TABLE 7: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITHOUT FORMALDEHYDE DEHYDROGENASE/NAD+

A. TEST ARTICLE: GPI 15715 (AQUAVAN®)
B. ASSAY NO.: 27276-0-431
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/29/2005
F. CELLS ANALYZED: 3×10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL ($\times 10^5$) Day 1	Day 2	Cumulative RSG[a]	AVG VC	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]	AVG VC	Relative Growth (%)[c]	Mutant Frequency ($\times 10^{-6}$)[d]
S9-Activation Controls[e]										
Vehicle Control	10.3	13.4	15.3		139	352	58.7		91.4	78.6
Vehicle Control	12.1	13.5	18.2		117	362	60.4		111.2	64.5
Vehicle Control	10.9	13.1	15.9	16.5	145	363	60.5	59.9	97.5	79.9
Formaldehyde Control 2.0 µg/mL	6.1	13.5	9.2		141	276	46.0		42.7	102.0
Formaldehyde Control 3.0 µg/mL	4.8	12.7	6.8		196	248	41.3		28.4	158.6[f]
MCA 2 µg/mL	7.3	9.2	7.5		317	179	29.8		22.6	354.9[f]
MCA 4 µg/mL	6.4	9.8	7.0		357	187	31.1		22.0	382.5[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
100	7.3	13.5	66.6		125	382	106.3		70.7	65.7
100	6.3	12.4	52.8		143	288	80.2		42.3	99.2
500	4.9	10.9	36.1		166	272	75.6		27.3	122.1
500	5.6	9.2	34.8		168	266	74.1		25.8	126.2
2500	1.9[g]	8.3	16.8		136	165	45.9		7.7	165.6[f]
2500	2.5[g]	8.4	17.0		141	188	52.2		8.9	150.0[f]
3400	3.5[g]	8.6	17.4		133	158	44.0		7.7	168.3[f]
3400	3.3[g]	8.0	16.2		161	149	41.6		6.7	216.1[f]

[a]RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2×10^{-1})
Decimal is moved to express the frequency in units of 10^{-6}
[e]Vehicle Control = 10% Water
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 148.7×10^{-6}
[g]Not subcultured

TABLE 8: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITHOUT FORMALDEHYDE DEHYDROGENASE/NAD+

A. TEST ARTICLE: GPI 15715 (AQUAVAN®)
B. ASSAY NO.: 27276-0-431
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/29/2005

		Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency (x 10^{-6})[d]		
Test Condition	Conc.	Day 1	Day 2	Abs %	Rel %	(%)	Total	Small	Large
Vehicle Control[e]									
	10%	92.8	93.2	58.7	98.1	91.4	78.6	62.5	16.1
	10%	109.0	110.3	60.4	100.8	111.2	64.5	50.6	13.9
	10%	98.2	96.4	60.5	101.1	97.5	79.9	64.9	15.0
Formaldehyde Control (µg/mL)									
	2.0	55.0	55.6	46.0	76.8	42.7	102.0	56.9	45.1
	3.0	43.2	41.2	41.3	68.9	28.4	158.6	110.1	48.5
MCA[f] (µg/mL)									
	2	65.8	45.4	29.8	49.8	22.6	354.9	178.0	176.8
	4	57.7	42.4	31.1	51.9	22.0	382.5	214.0	168.4
Test Article (µg/mL)									
	100	65.8	66.6	63.6	106.3	70.7	65.7	40.6	25.1
	100	56.8	52.8	48.0	80.2	42.3	99.2	56.8	42.4
	500	44.1	36.1	45.3	75.6	27.3	122.1	64.3	57.8
	500	50.5	34.8	44.4	74.1	25.8	126.2	70.5	55.7
	2500	27.0	16.8	27.5	45.9	7.7	165.6	98.0	67.5
	2500	27.0	17.0	31.3	52.2	8.9	150.0	90.7	59.3
	3400	27.0	17.4	26.4	44.0	7.7	168.3	107.6	60.7
	3400	27.0	16.2	24.9	41.6	6.7	216.1	128.5	87.6

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = Water
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

TABLE 9: INITIAL MUTATION ASSAY WITH ACTIVATION WITH FORMALDEHYDE DEHYDROGENASE/NAD+

A. TEST ARTICLE: GPI 15715 (AQUAVAN®)
B. ASSAY NO.: 27276-0-431
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/01/2005
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^5) Day 1	Day 2	Cumulative RSG[a]	AVG VC	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]	AVG VC	Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
S9-Activation Controls[e]										
Vehicle Control	8.7	13.9	13.4		89	440	73.3		69.6	40.7
Vehicle Control	8.8	14.9	14.6		92	592	98.7		101.6	30.9
Vehicle Control	10.5	15.5	18.1	15.4	69	626	104.4	92.1	133.4	22.0
Formaldehyde Control 2.0 µg/mL	7.5	14.8	12.3		119	516	86.0		74.9	46.1
Formaldehyde Control 3.0 µg/mL	5.2	14.0	8.1		93	504	84.0		48.0	36.8
MCA 2 µg/mL	4.5	10.9	5.5		422	348	58.0		22.3	242.6[f]
MCA 4 µg/mL	4.2	11.3	5.3		440	329	54.9		20.5	266.9[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
100	8.2	13.6	80.7		75	616	111.5		89.9	24.4
100	4.4	13.4	42.6		76	609	110.1		47.0	25.1
500	8.5	14.5	89.1		99	569	103.0		91.8	34.9
500	7.1	14.7	75.5		93	561	101.4		76.6	33.1
1000	4.9	13.3	47.1		73	451	81.5		38.4	32.4
1000	5.1	11.4	42.0		93	632	114.3		48.1	29.4
3400	6.7	12.8	62.0		117	557	100.9		62.6	41.9
3400	5.7	11.4	47.0		55	562	101.6		47.8	19.4

[a]RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e]Vehicle Control = 10% Water
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 62.4 x 10^{-6}

Study title: Study 3000-15715-00-12G: *In Vivo* Mouse Micronucleus Assay with GPI 15715

Key findings

- GPI 15715 was tested in the mouse in vivo bone marrow micronucleus assay at concentrations of 50, 100 and 150 mg/kg. Under the conditions of the study, GPI 15715 was concluded to be negative in the induction of the micronucleus formation in mice.
- GPI 15715 at higher dose (200 mg/kg) induced toxicity, 4/6 males died immediately after dosing. In the remaining two animals, polypnea and abnormal

postures were noted. Based on the clinical observation the dose selection appeared to be valid.

Study number: 3000-15715-00-12g
Volume # and page #: eCTD; Pages 1-23
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: August 21, 2000
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: Drug: GPI 15715; Lot #21708-21-3; Purity: 84.8%

Methods:

Species: [redacted] CD-1®(ICR) BR mice (6 males/dose/time point) b(4)

Doses used in definitive study: 50, 100, and 200/150 mg/kg

Basis of dose selection: A dose range finding study was conducted. The range-finding experiment demonstrated that an intravenous dose of 250 mg/kg was lethal in both males and females (1/1 male; 1/1 female).

Negative controls: The negative control used for the micronucleus assay was 0.9% sodium chloride.

Positive controls: The positive control used for the micronucleus assay was cyclophosphamide (25 mg/kg) dissolve in sterile water. The positive control utilized in this study is valid.

Incubation and sampling times: Bone marrow sampling took place at 24 and 48 hours post-dosing with the exception of the positive control group (at 24 hours only). The mice were administered with a single dose of the test article.

Results:

Study validity: The study is deemed valid for the following reasons: 1) previous pharmacokinetic assessments demonstrated systemic exposure, 2) dosing appeared to be adequate based upon the results of the dose-ranging study, 3) preparation and administration of the test substance was acceptable, 4) the species and number of animals/sex/group were acceptable, 5) tissue sampling and analysis was acceptable, 6) positive controls exhibited appropriate responses, and 7) the proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

Study outcome: The test article at 50, 100 and 150 mg/kg dose induced clinical signs of toxicity such as ataxia, lateral recumbent, and polypnea immediately post dosing. No such findings were noted one hr post dosing. At higher dose (200 mg/kg) 4/6 males died immediately after dosing. In the remaining two animals, polypnea and abnormal postures

were noted. There was no increase in the micronucleated polychromatic erythrocytes in the mouse bone marrow. This is in concurrence with the Sponsor's conclusions.

2.6.6.5 Carcinogenicity

There was no carcinogenicity studies conducted for this application. Carcinogenicity assessments are not required for the current acute indication of the test article.

2.6.6.5 Reproductive and developmental toxicology

Fertility and Early Embryonic Development

Study Title: Intravenous Fertility and General Reproduction Toxicity Study of GPI 15715 (AQUAVAN®) in Rats

Key study findings

- The male fertility was studied by intravenous administration of GPI 15715 in 25 males/treatment (0, 5, 10, and 20 mg/kg) group for 4 weeks prior to mating followed by 3 weeks of the cohabitation period. The female fertility was studied by intravenous administration of GPI 15715 in 25 females/treatment (0, 5, 10, and 20 mg/kg) group for 2 weeks prior to mating followed by 3 weeks of the cohabitation period and 1 week of gestation period. The dams were sacrifice at GD 13. The male and female fertility study designs are according to the OECD protocols and appeared to be valid.
- Female and male NOAEL=10 mg/kg/day based on clinical signs (ataxia, and decreased motor activity at 20 mg/kg, and > 10% decrease in body weight gain at 20 mg/kg). In this study the frank toxicity for the male and female was established based on the decrease in the body weight gain and clinical signs.
- There was a decrease in the sperm count (15%) and sperm-density (18%) at high dose, based on this finding, the NOAEL for the male fertility is established to be 10 mg/kg ($AUC_{0\text{-}inf}$ =6.5 mcg.h/mL). The Sponsor believed that the decrease in sperm count is not statistically significant and therefore, not test article related. All other male fertility parameters such as number of days of cohabitation, rats that mated, fertility index, and rats pregnant/rats in cohabitation were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day. Therefore, the Sponsor's NOAEL for the male fertility was >20 mg/kg.
- There were an increase in the number of nonviable embryos (2-3 folds) at all doses, the finding was observed in all treatment groups. Based on this finding; the NOAEL for the female fertility is established to be < 5 mg/kg. The Sponsor believed that the increase in nonviable embryos are not dose-related, therefore, the Sponsor's NOAEL for the female fertility was >20 mg/kg. All other female

fertility parameters such as number of day of cohabitation, copulatory index, fecundity index, and fertility index were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day.

- The toxicokinetic analyses were conducted at Days 1, 14, and 28 from the male rats only. There was a dose proportional increase in the exposure of GPI 15715 and propofol as indicated by the increase in the Cmax and AUC. The systemic exposures of propofol (27-90% ↓) and GPI 15715 (12-31% ↓) decreased indicating accumulation at Day 28. The half life of propofol and GPI 15715 were however comparable following single and repeat dose administration of GPI 15715.

Study number: 1707-007
Volume # and page #: eCTD submission; Page 1-727
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: August 9, 2003
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: Drug: GPI 15715, 176I0603, 96.4%

Methods:

Doses: 0, 5, 10, and 20 mg/kg/day
Species/strain: Rat/[redacted]:CD® (SD) IGS BR VAF/Plus® b(4)
Number/sex/group: 25/sex/group
Route, formulation, and volume: The drug product was administered intravenously by a slow bolus injection. The drug product formulation consisted of 35 mg/mL fospropofol in 10 mM tromethamine (TRIS), 0.25% monothioglycerol (MTG). The drug product was prepared using 0.9% saline for intravenous injection. The vehicle information is provided as follows (refer to **Sponsor's table).**

Vehicle Component	Description	Lot Number	Date Received	Storage Conditions	Expiration Date
Tromethamine, USP	White powder	QH0215	07 AUG 03	Room temperature	N/A[a]
Monothioglycerol, NF	Clear, colorless liquid	RA 0617EC75	07 AUG 03	Room temperature	JAN 08
Sterile Water for Injection, USP[b]	Clear, colorless liquid	J2E623	23 DEC 02	Room temperature	NOV 04
0.9% Sodium Chloride Injection, USP[b]	Clear liquid	J2H624	01 APR 03	Room temperature	DEC 04

N/A = Not Applicable
a. Assumed stable for the duration of the study.
b. Received from [redacted] b(4)

Satellite groups used for toxicokinetics: 12/sex group
Study design: The male rats were administered with the test article once daily beginning 28 days before cohabitation, through cohabitation (maximum 21 days) and continuing through the day before sacrifice. The female rats were administered with the test article once daily beginning 15 days before

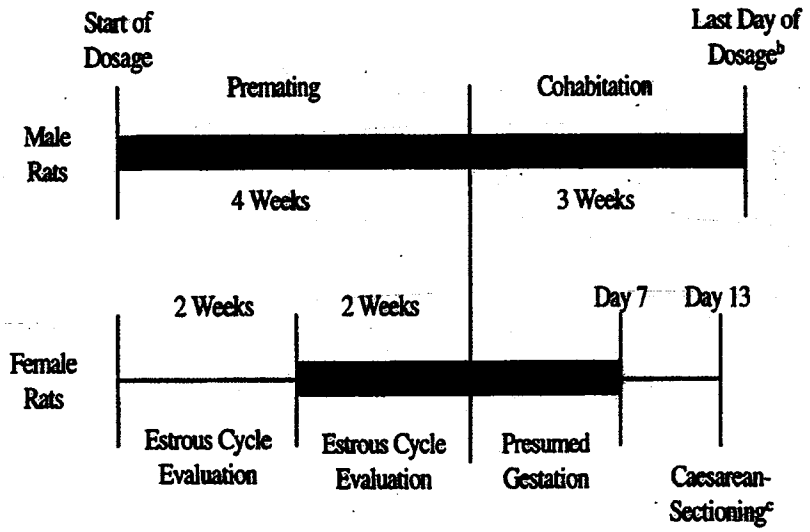
cohabitation, through cohabitation (maximum 21 days) and continuing through day 7 of presumed gestation. Following **is the Sponsor's study design table and** the dosing schedule.

Study design table:

Dosage Group	Dosage[a,b] (mg/kg/day)	Concentration (mg/mL)	Number of Rats per Sex	Assigned Numbers		
				Male Rats (Main Study)	Male Rats (Toxicokinetic Study)	Female Rats
I	0 (Vehicle)	0	25	15601 - 15625	N/A	15801 - 15825
II	5	0.5	25 + 12[c]	15626 - 15650	4339 - 4348, 12421[d], 4350	15826 - 15850
III	10	1	25 + 12[c]	15651 - 15675	16277 - 16282, 12422[e], 16284 - 16288	15851 - 15875
IV	20	2	25 + 12[c]	15676 - 15700	16289 - 16300	15876 - 15900

N/A = Not Applicable.

a. The test article was considered 100% pure for the purposes of dosage calculations.
b. The dosage volume was 10 mL/kg.
c. Male rats assigned to toxicokinetic evaluation.
d. Rat 4349 was found dead during blood collection on day 1 of study, prior to dosage administration, and was replaced with rat 12421.
e. Rat 16283 was found dead during blood collection on day 1 of study, prior to dosage administration, and was replaced with rat 12422.



Parameters and endpoints evaluated: The parameters evaluated in this study were mortality, clinical signs, body weight and food consumption, gross pathology of adults, reproductive organ weights and histopathology. Female specific data includes estrus cycles, time to insemination, number of corpora lutea, implantations, live embryos, and resorptions. Male specific data include sperm evaluation (concentration and motility).

Results:

Mortality: The animals were observed for viability at least twice each day of the study. One male rat was found dead at Day 20 of the study. The cause of death was attributed to trauma sustained when the rat attempted to free its teeth which were entangled in the home cage. The clinical signs associated with the trauma were swollen snout and red perinasal substance on Day 20. All of the tissues from this animal were appeared normal at necropsy.

Clinical signs: Observations for the test article related clinical signs were made daily before the dosage administration and approximately 10 mins and 60 mins post dosing. All rats at 20 mg/kg were observed with either ataxia or decreased motor activity immediately post dosing ($p \leq 0.01$). Additionally, significant increase ($p \leq 0.01$) in the impairment of the righting reflex was also observed in the high dose group animals. All of these clinical signs were noted at 10 mins post dosing and were not present at 60 mins post dosing period. The clinical signs of ataxia, impaired righting reflex, and decreased motor activity were also noted in some of the animals from the mid dose group, however, the incidence of such observations were much lower in the mid dose group animals. The clinical sign findings are considered test article related and might be associated with the exaggerated pharmacology of the compound.

Body weight: The body weight for males and females were recorded daily during the dosage and post dosage periods and at sacrifice. There was a significant ($p \leq 0.01$) decrease (18%↓) in the body weight gain after the first 28 days of dosing in the high dose group males compared to those of the controls. The body weight gain in the high dose group males continue to decrease during the cohabitation period and at necropsy, at Day 49, there was a 20% decrease in the body weight gain in the high dose group males compared to those of the control animals. The body weight gain in the high dose group females were also found to be significantly ($p \leq 0.01$) reduced (31%) prior to cohabitation at Day 15, compared to those of the control animals. The body weight gains were comparable between all of the dose groups during pregnancy.

Food consumption: The food consumption values for male and female rats were recorded weekly until cohabitation. In addition, food consumption was recorded during the gestation days 0, 7, 8, 10, and 13. There was slight decrease in food consumption in males prior to cohabitation but no such changes were observed in males during the cohabitation period. There were no changes in the food consumption in female rats.

Gross Necropsy Observations: All male rats were sacrificed at Day 49; there were no statistically significant changes in the reproductive organ weights in males (testes, seminal vesicle, prostate, and epididymis). All females were sacrificed at Day 13 of presumed gestation (that is seven days after the dosing was discontinued). There was no test article related changes in the gross necropsy observation in the dams.

Summary of Mortality, Clinical, and Necropsy Observations:

Parameters	**Dosages (mg/kg/day)**	
	Male	**Female**

	0	5	10	20	0	5	10	20
Mortality	-	-	-	1/25	-	-	-	-
Clinical Observations (number of animals showing incidence/ total number of animals observed)								
Ataxia	-	-	2/25	25/25	-	-	5/25	25/25
Decreased motor activity	-	-	2/25	25/25	-	-	1/25	25/25
Impaired righting reflex	-	-	1/25	25/25	-	-	-	25/25
Body weight Gain (mg/kg/day)								
Days 1-8	22	22	25	14; 34% ↓	5	5	6	2; 40% ↓
Days 1-49 M Days 1-15 F	142	133	148	113; 20% ↓	16	17	18	11; 31% ↓
Relative Food Consumption (g/kg/day)								
Days 1-15	70	65	68	66; 7% ↓	18	18	18	18
Days 1-28 M Days 1-15 F (last value recorded before cohabitation)	66	66	66	65	18	18	18	17

Toxicokinetics:

The toxicokinetic analyses were conducted at Days 1, 14, and 28 from the male rats only. There was a dose proportional increase in the exposure of GPI 15715 and propofol as indicated by the increase in the Cmax and AUC. The systemic exposures of propofol (27-90%, ↓) and GPI 15715 (12-31%, ↓) decreased indicating accumulation at Day 28. The half life of propofol and GPI 15715 were, however, comparable following single and repeat dose administration of GPI 15715.

Text Table 1. Summary Plasma GPI 15715 Toxicokinetic Parameters Following Single and Repeated IV Bolus Dosages of 5, 10 and 20 mg/kg/day to Male Rats on DSs 1, 14 and 28

Dose (mg/kg)	DS	AUCt (ng·h/mL)	AUCinf (ng·h/mL)	$AUC_{\tau(0-\tau)}$ (ng·h/mL)	Acc. Ratio*	Cmax** (ng/mL)	t½ (h)	AUC/ Dose	Cmax/ Dose
5	1	4659	4667	NA	NA	49333	0.21	932	9867
10	1	7394	7407	NA	NA	69067	0.20	739	6907
20	1	14763	14809	NA	NA	137667	0.23	738	6883
5	14	NA	NA	3474	0.746	34067	0.22	695	6813
10	14	NA	NA	6136	0.830	60867	0.16	614	6087
20	14	NA	NA	10524	0.713	94533	0.66	526	4727
5	28	NA	NA	3216	0.690	23000	0.28	643	4600
10	28	NA	NA	6470	0.875	54133	0.20	647	5413
20	28	NA	NA	10468	0.709	84700	0.21	523	4235

* Accumulation Ratio was calculated as $AUC_{\tau(0-\tau)}$ (DSs 14 or 28)/AUCt (DS 1) for all dose levels.
**Observed value following the end of the slow bolus injection.
NA Not Applicable

Text Table 2. Summary Plasma Propofol Toxicokinetic Parameters Following Single and Repeated IV Bolus Dosages of 5, 10 and 20 mg/kg/day to Male Rats on DSs 1, 14 and 28

Dose (mg/kg)	DS	AUCt (ng·h/mL)	AUCinf (ng·h/mL)	$AUC_{SS\,(0\text{-}\tau)}$ (ng·h/mL)	Acc. Ratio*	Cmax** (ng/mL)	t½ (h)	AUC/ Dose	Cmax/ Dose
5	1	122	126	NA	NA	310	0.36	24.4	62.1
10	1	355	357	NA	NA	996	0.74	35.5	100
20	1	724	747	NA	NA	1770	1.16	36.2	88.5
5	14	NA	NA	186	1.52	711	0.46	37.2	142
10	14	NA	NA	473	1.33	1547	1.00	47.3	155
20	14	NA	NA	922	1.27	2917	1.00	46.1	146
5	28	NA	NA	231	1.89	710	1.28	46.2	142
10	28	NA	NA	531	1.50	1417	1.03	53.1	142
20	28	NA	NA	1071	1.48	2870	1.14	53.6	144

* Accumulation Ratio was calculated as $AUC_{ss\,(0\text{-}\tau)}$ (DS 14 or 28)/AUCt (DS 1) for all dose levels.
**Observed value following the end of the slow bolus injection.
NA Not Applicable

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

All mating and fertility parameters for males such as number of days of cohabitation, rats that mated, fertility index, and rats pregnant/rats in cohabitation were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day. However, there was a dose related decrease (not statistically significant) in the sperm count (16% ↓) and sperm density (18% ↓) in the caudal epididymis of the high dose males compared to those of the control animals. A complete cycle of spermatogenesis occurs in the rat within 10 weeks which can not be measured with the male fertility study design. Sperm counts, motility, and morphology have been used traditionally to characterize the fertility parameters in males. Sperm mature during transport to the epididymis; therefore, epididymal sperm are preferred for analysis. The decrease in the sperm count and density observed in this study might indicate a test article related deficiency in the sperm maturation at 20 mg/kg/day. The treatment related decrease in the sperm count and sperm density is considered biologically relevant because the findings are observed to be dose dependence.

All mating and fertility parameters for females such as number of day of cohabitation, rats that mated, copulatory index, fecundity index, and fertility index were unaffected by dosages of GPI 15715 as high as 20 mg/kg/day. However, there was a non-statistically significant, not dose-dependent, increase in the number of nonviable embryos around the preimplantation period. There was approximately 2-3 fold increase in the nonviable embryos at all dose groups. The number of nonviable embryos does not directly correlate with the preimplantation loss. Considering that the animals were treated up to GD 7 and the dams were sacrificed at GD 13, the increase in the nonviable embryos observed at necropsy might be resulted from the delayed test article related effect.

Summary of Mating and Fertility Observations in Males and Females:

Parameters	Dosages (mg/kg/day)			
	0	5	10	20
Mating and Fertility observation /Females:				
Estrus Cycle Observations				
Pre dosing estrus cycling/Rats with 6 or more days of diestrous (N)	0	0	0	0
Pre cohabitation estrus cycling/Rats w6 or more days of diestrous (N)	0	1	1	1
C-Section Observations				
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 ↑)
Mating and Fertility observation /Males:				
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%↓

Embryo-Fetal Development

Study Title: Intravenous Developmental Toxicity Study of GPI 15715 in Rats

Key study findings

- The embryofetal development was studied in rats by administering (IV) 0, 5, 20, and 45 mg/kg/day of the test article in 25 time pregnant dams/group from GD 7-17, the dams were sacrificed at Day 21.
- The maternal toxicity was indicated by mortality in the high dose group (2/25), and clinical signs of ataxia and decreased motor activities in all animals from the mid and high dose group. Based on these findings NOAEL for maternal toxicity was established as 5 mg/kg. This is **in concurrence with Sponsor's NOAEL.**
- There was an increase in the number of dams with resorptions. The percent of dams with any resorptions were 33, 52, 48, and 44 with 0, 5, 10, and 20 mg/kg/day dose group.
- There was an increase in the number of fetus with asymmetric sternal centra and wavy ribs in the treated animals. These variations are believed to be related to the incomplete ossification. There were no such changes in the concurrent controls. In addition, there was an additional central ribs 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. Because of the increase in this incidence compare to that of the control, this finding in considered as test article related. The NOAEL for fetal variations in this study **could not be established. The Sponsor's NOAEL for fetal** variation is >45 mg/kg/day.

Study number: 3000-15715-01-05g
Volume # and page #: eCTD submission; Page 1-253
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: May 24, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: Drug: GPI 15715; Lot #:12638-D2-01-001; Purity: 96.0%

Methods:

Doses: 0, 5, 20, and 45 mg/kg/day
Species/strain: Rat/[redacted]CD® (SD) IGS BR VAF/Plus®
Number/sex/group: 25 timed pregnant females/group
Route and formulation: The drug substance was administered intravenously by a slow bolus injection. The drug substance was formulated in 0.9% saline, and the saline was used as vehicle control.
Satellite groups used for toxicokinetics: 9 females/group
Study design: The test articles or vehicle were administered intravenously once daily to rats GDs 7 through 17 (refer to **the attached Sponsor's study design** table). The dams were sacrificed at GD 21.

b(4)

Study design table:

Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Rat Numbers
I	0 (Vehicle)	0	10	25	6701 - 6725
II	5	0.5	10	25 + 9[b]	6726 - 6750, 5331 - 5353[b], 4293[b], 12800[b], 5356 - 5359[b]
III	20	2.0	10	25 + 9[b]	6751 - 6775, 5360 - 5362[b], 4297[b], 5364 - 5367[b], 3980[b]
IV	45	4.5	10	25 + 9[b]	6776 - 6800, 5369 - 5377[b]

a. The test article will be considered 88.2% pure for the purpose of dosage calculations.
b. Nine rats were assigned for toxicokinetic analysis.

Parameters and endpoints evaluated: The parameter evaluated were clinical signs, body weight (daily), feed consumption, gross necropsy, reproductive parameters (corpora lutea, implantations, uterus/placental weight, early/late resorptions, viable fetuses, sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations).

Results:

Mortality: The animals were observed for viability at least twice each day of the study. In the 45 mg/kg/day dose group 2 dams were found dead, one dam died at GD12 (23

mins post 6th dose and the other dam died at GD13 (29 mins post 7th dose). The clinical signs observed in both of these dams were anesthesia and sedation prior to death, all tissue appeared normal at necropsy. The dam which died at GD12 lost approximately 40% of the body weight. The embryos appeared normal for their developmental ages.

Clinical signs: Observations for the test article related clinical signs were made daily before the dosage administration and approximately 10 mins and 60 mins post dosing. All rats in the mid and high dose group had ataxia, decreased motor activity, and were observed to be anesthetized on most of the days (8 days onwards). Additionally, impaired righting reflex occurred in significant ($p \leq 0.01$) numbers.

Body weight: The body weights of females were recorded daily during the dosage and post dosage periods and at sacrifice. There was a significant change in the body weight gains at mid (33% ↓) and high (40% ↓) dose dams compared to the body weight of the vehicle control dams. The decrease in body weights might be partially due to the decrease in the feed consumption.

Food consumption: The food consumption values for male and female rats were recorded daily until the dams were sacrificed. There was a slight change in the food consumption in animals from the mid and high dose group dams (approximately 6% ↓) compared to the feed consumption of the vehicle control dams.

Summary of Findings from Dams/ Embryo-Fetal Development Study:

Parameters	**Dose (mg/kg/day)**			
	0	**5**	**20**	**45**
Mortality	0	0	0	2
Clinical Observation				
Sedation	0	0	++	+++
Decreased motor activity	0	0	++	++
Lost of righting reflex	0	0	+	++
Body Weight Changes (g) mean ± sd; (Days 7-21)	+28 ± 12	+30 ± 14	+19 ± 12 33%↓	+17± 14 40%↓
Food Consumption (g/day) mean ± sd; (Days 7-21)	23 ± 1.4	23 ± 1.8	21 ± 2.2 6% ↓	21 ± 2.0 6% ↓
Dams with resorptions	33%	52%	48%	44%

+ Minimal
++ Mild
+++ Moderate

Toxicokinetics:

The toxicokinetic analyses were conducted at Days 7 and 18 from the dams. There was a dose proportional increase in the exposure of GPI 15751 and propofol as indicated by the increase in the Cmax and AUC. The systemic exposures of propofol and GPI 15715 decreased indicating accumulation at Day 17.

Summary of toxicokinetic analyses:

Parameters	Dose (mg/kg/day)			
	0	5	20	45
Toxicokinetics/ GPI 15715				
Cmax (µg/mL) GD7	NT	5.3	18	47
AUC $_{0-t}$ (µg.h/mL) GD7	NT	99	373	935
Cmax (µg/mL) GD17	NT	1.6	7	26
AUC $_{0-t}$ (µg.h/mL) GD17	NT	29	170	477
Toxicokinetics/ Propofol				
Cmax (µg/mL) GD7	NT	0.23	1.5	6
AUC $_{0-t}$ (µg.h/mL) GD7	NT	6	61	240
Cmax (µg/mL) GD17	NT	0.34	3	10
AUC $_{0-t}$ (µg.h/mL) GD17	NT	18	109	411

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, and percent live male fetuses were comparable among the four dose groups. However, the percent of dams with any resorptions increased in all test articles treated group. This finding although not statistically significant, is considered biologically relevant. This is because of the fact that the findings were observed at higher percent compared to those of the control in all treatment groups.

Offspring (malformations, variations, etc.):

There were no fetal soft tissue malformations. There were two fetuses from mid dose group with folded retinas which were considered as variations. There was a cervical rib at the 7th cervical vertebra in one fetus in the vehicle control group. This incidence increased in the entire test article treated groups (2-3.5 folds, ↑). Wavy ribs were present in 1 and 3 fetuses from the 20 and 45 mg/kg/day dose group; no such changes were noted in the experimental control group. Two of the three fetuses in the high dose group with wavy ribs also had hypoplastic ribs. One fetus in the 5 mg/kg/day group and one fetus in the 20 mg/kg/day group had asymmetric sternal centra, one fetus in the 45 mg/kg/day group had incompletely ossified sternal centra. No such changes were noted in the fetuses from the vehicle control group.

Summary of Findings from Rat Embryo-Fetal Development Study:

Parameters	Dose (mg/kg/day)			
	0	5	20	45
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%)
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%)
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%)

L = Litter; F = Fetus

Study title: Intravenous Developmental Toxicity Study of GPI 15715 in Rabbits

Key study findings

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

- One of the major changes in the nasal area in skull was the displaced midline suture. The percent increase in the displaced midline suture in the 0, 14, 28, 56, and 70 mg/kg/day dose group were 10, 16, 16, 18, and 25 respectively.
- The malformations of the thoracic vertebrae were observed in two fetuses. One fetus in the 14 mg/kg/day dose group had a right hemi vertebra as a 9th arch; this fetus also had centrum with attached rib. Another fetus in the high dose group, 70 mg/kg/day had a small arch in the left 11th, and fused right 12 and 13th right thoracic ribs and short left 11th rib.
- The NOEL for the fetal findings were established to be <14 mg/kg/day based on the above mentioned findings. This is in **contrast to the Sponsor's NOEL of >70** mg/kg/day based on the non dose related findings of the malformations and variations. According to the reviewer, some of the variations such as displaced midline suture, angulated hyoids, and wavy ribs are dose related. The gross external alterations are associated with malformations in the skull, vertebrae, and soft tissues and were not observed in the concurrent controls, therefore considered as test article related.

Study number: GPI 3000-15715-01-04G
Volume # and page #: eCTD submission; Pages 1-302
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 07-06-2001
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: Lot #: 12164-GI-00-001; Purity: 98.7%

Methods:

Doses: 0, 14, 28, 56, and 70 mg/kg/day
Species/strain: Rabbit/Hra: (NZW) SPF
Number/sex/group: 20 timed mated female rabbits/group
Route and formulation: Intravenous; 0.9% sterile water.
Satellite groups used for toxicokinetics: 3/timed mated female rabbits/group. On GD 6 and 18, blood samples were collected prior to dosing and 1, 30, 60, mins, 8 hr, and 24 hrs after dosing from each of these rabbits for toxicokinetic analysis.
Study design: The female rabbits were naturally bred and mated with breeder male rabbits, prior to the shipment to the [redacted] The day of mating was considered GD 0. These timed mated females were administered IV with the vehicle or the test article from GD 6-18, the animals were sacrificed on GD 29. b(4)
Following is the Sponsor's study design table.

Study design table:

Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers	
					Main Study	Toxicokinetic Study[b]
I	0	0	1	20	6501 - 6520	
II	14	14	1	20 + 3[b]	6521 - 6540	6581 - 6583
III	28	28	1	20 + 3[b]	6541 - 6560	6584 - 6586
IV	56	56	1	20 + 3[b]	6561 - 6580	6587 - 6589
V	70	84	.83	20 + 3[b]	6481 - 6483, 4274[c], 6485 - 6489, 5200[c], 6491, 6492, 6480[c], 6494 - 6500,	2798 -2800

a. The test article was considered 93.3% pure for the purpose of dosage calculations.
b. Additional rabbits assigned for toxicokinetic analysis.
c. Rabbits 6484, 6490 and 6493 were found dead after dosage administration on DG 6 of 84 mg/kg/day and were replaced with 4274, 5200 and 6480, respectively.

Observation and results

Mortality (dams): All rabbits were observed for viability twice daily. There was a dose related increase in the mortality in this study. The number of does died or found moribund at 14, 28, 56, and 70 mg/kg/day were 1, 1, 2, and 6 respectively. One doe in the 14 mg/kg/day dose group was moribund sacrificed on GD 22. Adverse clinical observations prior to sacrifice include ataxia, lacrimation, swollen eyelids, ungroomed coat, swollen front and hind paws, dehydration, decreased feed consumption, and body weight. One doe in the 28 mg/kg/day dose group died on GD 28. The adverse clinical observations were ataxia, bradypnea, decreased motor activity, impaired righting reflex, reduced feed consumption and body weight. There were two deaths in the 56 mg/kg/day dose group within 10 mins post dosing at GD 12 and GD 14. The adverse clinical signs observed were ataxia, decreased motor activity, excess salivation, nystagmus, and bradypnea. These two does gained weight and had normal food consumption prior to death. There were two deaths in the 70 mg/kg/day dose group within 10 mins post dosing at GD 6, one of these two does received 84 mg/kg dosing according to the original protocol. The four other does in the 70 mg/kg/day dose group died with 20-40 mins post dosing at GDs 0-18. All of these does showed clinical signs of ataxia, decreased motor activity, excess salivation, nystagmus, bradypnea, and loss of righting reflex. In addition, two of these does also had swollen ears. All of these does gained weight and had normal food consumption. All tissues examined at necropsy appeared normal, and all of the conceptuses appeared normal for their developmental ages.

Clinical signs (dams): The rabbits were examined for clinical signs, abortions, premature deliveries, and deaths before dosing and approximately 10 and 60 mins after the administrations of the test article. The clinical signs including ataxia, impaired righting reflex, and decrease motor activity was observed in all test article treated animals. The clinical signs of sedation resulting from the administration of the test article appeared to be dose related as regards to the depth of the sedation (refer to table below). All of these above mentioned clinical signs are considered to be due to the exaggerated pharmacology of the test article. The clinical signs were observed at 10 mins post dosing but were not apparent at 60 mins post dosing suggesting complete recovery.

Body weight (dams): The body weights were recorded on GD 0 and daily during the dosage and post dosage period. The absolute body weight and body weight gains were comparable among the five dosage groups for the entire dosing and post dosing period.

Food consumption (dams): The food consumptions were recorded daily throughout the study. There was a statistically significant reduction in the feed consumption between GDs 15-19 in 70 mg/kg/day dose group. As this reduction occurred in the highest dose group, it was considered test article related.

Terminal and Necropsy Evaluation, Does:

Parameter	Dose (mg/kg/day)				
	0	14	28	56	70
Mortality		1	1	2	6
Clinical Observation					
Sedation	-	+	++	+++	+++
Swollen ear	-	-	-	-	+++
Salivation	-	-	-	++	++
Body Weight (kg)	4.0	↓1.5	↓0.75	↓1.25	↓0.25
Food Consumption (g/d)	171	↓3.0	↓0.6	↓4.3	↓6.4

+ Minimal
++ Mild
+++ Moderate

Toxicokinetics:

The toxicokinetic analyses were conducted at GDs 6 and 18. There was a dose proportional increase in the exposure of GPI 15751 and propofol as indicated by the increase in the Cmax and AUC. The systemic exposures of propofol decreased at GD 18 compare to that of GD 6 indicating accumulation at GD 18.

Summary of toxicokinetic analyses:

Parameter	Dose (mg/kg/day)				
	0	14	28	56	70
Toxicokinetics					
GPI 15715					
Cmax (µg/mL) GD 6	NT	2.5	17.5	96	165
AUC $_{0\text{-}t}$ (µg.h/mL) GD 6	NT	55	307	1656	312
Cmax (µg/mL) GD 18	NT	4.6	14.6	48	ND
AUC $_{0\text{-}t}$ (µg.h/mL) GD 18	NT	76	242	803	ND
Propofol					
Cmax (µg/mL) GD 6	NT	0.3	1.3	5.8	115
AUC $_{0\text{-}t}$ (µg.h/mL) GD 6	NT	9.7	282	114.3	216
Cmax (µg/mL) GD 18	NT	0.5	1.7	4.1	ND
AUC $_{0\text{-}t}$ (µg.h/mL) GD 18	NT	11.6	250	76.3	ND

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.) and Offspring (malformations, variations, etc.):

The C-sectioning and litter parameters such as corpora lutea, implantation sites, litter sizes, palcentae, fetal body weight, and resorptions were unaffected by the test article administration. However, it was noted that 1 (no female fetuses), 1 (no male fetuses), and 2 does (one with no male and the other with no female fetuses) in the 14, 28, and 56 mg/kg/day does group respectively had either no male or no females fetuses. Also, the percent of the live male fetuses/litter decreased with the higher dose groups (% lives male fetus/litter in 0, 14, 28, 56, and 70 mg/kg/day were 54, 47, 43, 38, and 40 respectively). There were no fetal malformations in the vehicle control group. However, 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 observations 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, fetus 6564-6 had meningocele in the head. Skeletal evaluation revealed incompletely ossified parietal and frontal bones in the skull.

The soft tissue variations were noted in one fetus (6531-2) in the 14 mg/kg/day dose group. This fetus had a circumcorneal hemorrhage in the right eye. The Sponsor believed that this might have occurred from trauma during processing.

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 rib.

There were several skeletal tissue variations in the fetuses from the test article treated does. Wavy ribs were present in one fetus (5200-9) in the 70 mg/kg/day dose group. The arches of the cervical vertebra were fused in one 28 mg/kg/day fetus (6560-7). Fused sternal centra occurred in one fetus (6525-2) from the 14 mg/kg/day dose group. One fetus in the 28 mg/kg/day dose group had an irregularly shaped ala within the scapulae. An angulated hyoid occurred in 1, 3, 11, 3, and 5 fetuses from the 0, 14, 28, 56, and 70 mg/kg/day dose groups. The increases in the 28 and 70 mg/kg/day dose group were significantly different ($p \leq 0.01$) from the vehicle control group. The increase was considered not test article related by the Sponsor because they were not dose dependent. There was a test article related increase in the irregular ossification in the skull including nasal area and the parietal and frontal bones. The percent increase in the total irregular ossification within the skull in the 0 and 70 mg/kg/day dose group were 45 and 68 respectively. The irregular ossification was noted in the nasal area and includes

internasal suture, intranasal suture, irregular suture, and displaced midline suture. The percent increase in the irregular ossification within the nasal area in the 0, 14, 28, 56, and 70 mg/kg/day dose group were 20, 26, 26, 35, and 33 respectively. One of the major changes in the nasal are 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 above mentioned variations were considered unrelated to the test article by the Sponsor because according to their analyses the findings were not dose related.

Terminal and Necropsy Evaluation, Offspring:

Parameter	Dose (mg/kg/day)				
	0	14	28	56	70
Mortality	0	1	1	2	6
Pregnancy	20	19	19	17	12
Number of Fetuses	168	148	148	119	96
Skeletal Alterations					
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, Intranasal suture, **Irregular suture'** 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%)
Skull/Irregular Ossification: **Nasal-contained an Internasal**	1L (5%) 1F (0.6%)	2L (11%) 2F (1%)	0L (0%) 0F (0%)	4L (23%) 6F (5%)*	0L (0%) 0F (0%)
Hyoid/Angulated	1L (5%) 1F (0.6%)	2L (11%) 3F (2%)	5L (26%) 11F (7%)	2L (12%) 3F (3%)	3L (25%) 5F (3%)
Caudal vertebrae/ misaligned	1L (5%) 1F (0.6%)	0L (0%) 0F (0%)	3L (16%) 4F (3%)	0L (0%) 0F (0%)	1L (8.3%) 1F (1%)
Skeletal Malformations					
Ribs short, fused, wavy 5th-7th	0L (0%) 0F (0%)	0L (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 (0%) 0F (0%)	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 (0.7%)	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 (0.7%)	1L (5.9%) 1F (0.8%)	1L (8.3%) 1F (1%)

L = litters; F = fetuses
* = statistically significant p>0.01

Prenatal and Postnatal Development

Study title: Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GPI 15715 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key study findings

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

Study number: 1707-006
Volume # and page #: eCTD submission; Pages 1-373
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 09-23-2003
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: Drug: GPI 15715, 176I0603, 96.4%

Methods:

Doses: 0, 5, 10, and 20 mg/kg/day
Species/strain: Rat/[redacted]:CD® (SD) IGS BR VAF/Plus® b(4)
Number/sex/group: 25/sex/group
Route, formulation, and volume: The drug product was administered intravenously by a slow bolus injection. The drug product formulation consisted

of 35 mg/mL GPI 15715 in 10 mM tromethamine (TRIS), 0.25% monothioglycerol (MTG). The drug product was prepared using 0.9% saline. The vehicle information is provided **below (refer to Sponsor's table).**

Study design table:

Vehicle Component	Description	Lot Number	Date Received	Storage Conditions	Expiration Date
Tromethamine, USP	White powder	QH0215	07 AUG 03	Room temperature	N/A[a]
Monothioglycerol, NF	Clear, colorless liquid	RA 0617EC75	07 AUG 03	Room temperature	JAN 08
Sterile Water for Injection, USP[b]	Clear, colorless liquid	J2E623	23 DEC 02	Room temperature	NOV 04
0.9% Sodium Chloride Injection, USP[b]	Clear liquid	J2H624	01 APR 03	Room temperature	DEC 04

N/A = Not Applicable
a. Assumed stable for the duration of the study.
b. Received from [redacted]

b(4)

Satellite groups used for toxicokinetics: There was no satellite group; toxicokinetics were not analyzed in this study.
Study design: The rats were administered the test article once daily from GD 7-LD 20 or GD 24. F_1 males or females were not dosed but were likely exposed in utero. It is unknown if drug is secreted into milk.
F_1 were reared to reproductive maturity.
Parameters and endpoints evaluated: F_0 were evaluated for clinical signs, body weight, feed consumption, pregnancy duration/parturition, and necropsy. F_1 were evaluated for number, viability, weight, physical development, sensory/motor functions learning/behavior/memory and fertility. F_2 were evaluated to ensure fertility of the F_1 offspring.

Results:

F_0 in-life

Mortality (dams): All rats were observed for viability twice daily. All female rats survived to scheduled sacrifice.

Clinical signs (dams): The rats were examined for clinical signs, abortions, premature deliveries, and deaths before dosing and approximately 10 and 60 mins after the administrations of the test article. All rats in the high dose group were observed with the clinical signs of ataxia, decreased motor activity, and impaired righting reflex during the gestation and lactation period. The clinican signs of ataxia also occurred in 10 rats in the 10 mg/kg/day dose group during the gestation period. Decreased motor activity occurred in one rat during the gestation period and impaired righting reflex occurred in two rats in the 10 mg/kg/day dose group. These clinical signs were observed at 10 mins post dosage and were not present at 60 mins post dosage in both of the 10 and 20 mg/kg/day dose groups.

Body weight (dams): Body weights were recorded on GD 0 and daily during the dosage and post dosage period. The body weight gain decreased (10%, ↓) significantly ($p \leq 0.01$) between GDs 0-20 in the 20 mg/kg/day dose group. The absolute body weight decreased in the high dose group throughout the dosing period; however, the body weight gains were comparable between the four dose groups during the lactation period.

Food consumption (dams): Food consumption was record on GDs 0, 7, 10, 12, 15, 18, 20, and 25 (if necessary) and LDs 1, 4, 7, 10, and 14). Relative food consumption decreased significantly ($p \leq 0.05$) between GDs 0-20 in the 20 mg/kg/day dose group. Absolute food consumption also decreased significantly ($p \leq 0.05$) between LDs 10-14 in the 20 mg/kg/day dose group. The absolute and relative food consumption values were unaffected by dosages of GPI 15715 in the 10 and 5 mg/kg/day dose groups.

Terminal and Necropsy Evaluation in F_0 Dams:

Parameter	Dose (mg/kg/day)			
	0	5	10	20
Mortality	0	0	0	0
Clinical Observations				
Ataxia	-	-	10/25	25/25
Decreased motor activity	-	-	1/25	25/25
Impaired righting reflex	-	-	2/25	25/25
Body Weight Gain(g) GDs 0-20	+127 ± 14	+129 ± 20	+124 ± 16 ↓4%	+115 ± 13 ↓10%
Food Consumption (g/kg/day); GDs 0-20	+ 67 ± 2	+ 68 ± 2	+ 67 ± 2	+65 ± 4 ↓3%
Body Weight Gain(g) LDs 1-21	+ 19 ± 14	+16 ± 11	+8 ± 22 ↓4%	+14 ± 13 ↓5%
Food Consumption (g/d) LDs 1-14	+48 ± 4	+48 ± 3	+48 ± 3;	+45 ± 4 ↓7 %

F_0 necropsy: Natural Delivery and Litter Observations: The natural delivery observations such as dams delivering litters, duration of gestation, implantation sites, gestation index, and number of dams with still born/live/born/pups dyeing were comparable among the four dose groups and did not differ significantly. The values for the lactation index, average body weights of pups, average live and still born pups, percent of male pups per number of pups sexed per litter size and weight were comparable among the four dose groups. One dam in the 10 mg/kg dose group had all litters died at LD 2. There was an increase in the number of pups died which between the LDs 1-14.

F_0: Natural delivery and Litter Observations

Parameter	Dose (mg/kg/day)			
	0	5	10	20
Mating/Fertility F_0				
Pregnant	24/25	25/25	21/25	24/25
Dams w/all pups dyeing	-	-	1	-

Dams died between D 4-14 (%)	**0.6**	**1.2**	**1.2**	**0.9**

F_1 Neonatal Observations: The pups were examined for clinical signs once daily after the administration of the test article. The clinical observations from birth to postpartum Day 21 of the F_1 generation pups were limited to scabs in ear, chest mass, chest scab, and nose scab in the high dose group. The biological significance of such findings is unknown.

F_1 Neonatal Observations:

Parameter	**Dose (mg/kg/day)**			
	0	**5**	**10**	**20**
F_1 Neonatal Parameters				
Pups mortality	**2**	**2**	**0**	**5**
Clinical Observations; Birth- LD 21				
Scab in ear				**1**
Head or neck: purple	**1**	**0**	**1**	**2**
Chest: mass & scab	**0**	**0**	**0**	**2**
Nose: scab	**0**	**0**	**0**	**1**

F_1 Postnatal Observations (Clinical signs, Body weight, Food consumption):
The F_1 male and female rats were examined for clinical signs once daily for clinical signs. The body weights and the feed consumption were recorded weekly throughout the experimental period. The male and female clinical signs include scabs in the back, chromodacryorrhea, excessive salivation, or misaligned or broken incisor, swollen ear, and soft and liquid feces. The body weights and the body weight gains during the pre cohabitation period for the F_1 males and females were unaffected by the administration of the test article to the F_0 generation dams as high as 20 mg/kg/day. Maternal body weight gains in the F_1 generation rats during gestation were also comparable in the four maternal dose groups. The male body weight changes, however, decreased significantly during the cohabitation period. Although not statistically significant, the finding is considered biologically relevant because of its dose dependence. There were no significant, biologically relevant changes in the food consumption in the F_1 males and females. Sexual maturation was unaffected by the maternal dosages as high as 10 mg/kg. The average day on which preputial separation or vaginal patency occurred was comparable among the dose groups and did not significantly differ. The mating and fertility parameters such as fertility index, days in cohabitation, the number of rats that mated, and the number of pregnancies per cohabitation were comparable among the four maternal dose groups in the F_1 generation males and females.

F_1 Post Natal Development:

Parameter	**Dose (mg/kg/day)**			
	0	**5**	**10**	**20**
F_1 Postnatal Parameters/ Reproductive Maturity/ Fertility Parameters				
Male Body weight	**+ 410 ± 42**	**+ 433 ± 47**	**+ 411 ± 52**	**+ 412 ± 27**

Days 1-91				
Female Body weight Days 1-65	**+ 221 ± 26**	**+ 220 ± 15**	**+ 229 ± 29**	**+ 220 ± 18**
Preputial separation (average days post partum that the prepuce separated)	**45.7 ± 2.6**	**45.8 ± 1.9**	**45.4 ± 2.4**	**45.0 ± 1.8**
Vaginal Patency (average days post partum that the vagina was patent)	**33.4 ± 2.4**	**33.4 ± 1.6**	**33.9 ± 2.5**	**34.2 ± 2.9**
Male Body weight Days 92-99 (cohabitation)	**+ 12 ± 5**	**+ 10 ± 8**	**+ 11 ± 5**	**+ 8 ± 12**
Female Body weight (Gestation)	**+ 175 ± 23**	**+ 180 ± 23**	**+ 176 ± 15**	**+ 178 ± 16**
Dams w/any resorptions N (%)	**9 (41%)**	**5 (20%)**	**9 (36%)**	**13 (54%)**

F_1 Behavioral development:

The F_1 males and females were evaluated for learning beginning 24 days postpartum by using the passive avoidance apparatus. In this study the animals were allowed to explore the brightly lighted compartment of the apparatus **until it entered the 'dark' compartment,** the sliding door separating the two compartments was then immediately closed and a brief pulse of current was delivered to the floor. The rat was then removed form the apparatus and returned to its cage for 30 mins prior to the next trial. The trials were repeated either for 15 times or until the rat remained in the bright compartment for 60 secs on two consecutive trials. The latency to enter the dark compartment or the maximum of 60 secs intervals were recorded for each trial and considered as the criteria for learning. The test sessions were repeated twice and were separated by a one week interval. Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session (to compare the overall learning ability), the latency to enter the dark compartment from the bright compartment on trial 1a in the first session (to compare the exploratory behavior), the latency to enter the dark compartment from the bright compartment on trial 2b in the first session (to compare the short-term retention), the latency to enter the dark compartment from the bright compartment on trial 1b in the second session (to compare the long term retention). There was a difference in the values for learning, short term retention as well as long term retention of response in the F_1 generation males but not in females as observed in the passive avoidance paradigm. The latency in (secs) for the males to enter the dark part of the passive avoidance compartment in the trial 1b of the session 1, in the 0, 5, 10, and 20 mg/kg were 20, 18, 14, and 14 respectively suggesting a dose related decrease in the short term retention of learning in males. Similarly, the latency in (secs) for the males to enter the dark part of the passive avoidance compartment in the trial 1b of the session 1, in the 0, 5, 10, and 20 mg/kg were 31, 30, 27, and 24, respectively, suggesting a dose related decrease in the long term retention of learning in males. These changes were not statistically significant, however, considered biologically relevant because of the dose dependence.

F_1 Behavioral development:

Parameter	Dose (mg/kg/day)			
	0	5	10	20
Passive Avoidance Test				
Male Rats: Session A				
Latency Trial 1b	4.2 ± 2.6	4.4 ± 3.1	4.3 ± 2.3	4.1 ± 2.8
Latency Trial 2b	20.7 ± 2.6	18.1 ± 19	13.7 ± 11.6	14.6 ± 15.3
Male Rats: Session B				
Latency Trial 1b	31.3 ± 24.2	30.7 ± 23.9	27.3 ± 25.8	24.2 ± 22.3

The F_1 rats were tested 70 day postpartum in an M water maze test for evaluating the learning and memory. Each rat was tested twice. In each test a maximum number of 15 trials were conducted. In each of the trials the rats were required to choose the correct goal) one arm of the M maize which was taught to them during the first few trials. The number of trials needed to reach the correct goal and the time required to reach the goal were counted at Day 1 of the test to evaluate the short term memory. The study was repeated on Day 2 to evaluate the long term memory. As indicated in the following table # C 17 from the Applicant, there were no dose dependent adverse changes in the short and long term learning and memory in the male and female rats.

PROTOCOL 1707-006: INTRAVENOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF GPI 15715 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION

TABLE C17 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	10	20
MALE RATS					
SESSION 1a	N	24	25	20	24
TRIALS TO CRITERION	MEAN±S.D.	8.2 ± 2.2	8.2 ± 2.4	9.6 ± 2.2	8.5 ± 2.8
ERRORS PER TRIAL	MEAN±S.D.	0.43 ± 0.29	0.38 ± 0.20	0.50 ± 0.32	0.40 ± 0.18
LATENCY TRIAL 2b	MEAN±S.D.	12.9 ± 5.4	16.3 ± 8.7	14.6 ± 8.0	15.5 ± 11.6
FAILED TO LEARN c	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
SESSION 2a	N	24	24	20	24
TRIALS TO CRITERION	MEAN±S.D.	6.3 ± 2.5	5.1 ± 0.3*	6.0 ± 1.5	5.8 ± 1.3
ERRORS PER TRIAL	MEAN±S.D.	0.12 ± 0.18	0.01 ± 0.05*	0.14 ± 0.16	0.09 ± 0.11
LATENCY TRIAL 1b	MEAN±S.D.	12.3 ± 9.5	8.6 ± 3.6	14.3 ± 8.3	12.6 ± 8.1
FEMALE RATS					
SESSION 1a	N	24	25	20	24
TRIALS TO CRITERION	MEAN±S.D.	8.5 ± 2.6	7.5 ± 1.4	8.6 ± 2.9	8.1 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.38 ± 0.18	0.33 ± 0.13	0.46 ± 0.38	0.37 ± 0.26
LATENCY TRIAL 2b	MEAN±S.D.	14.2 ± 12.0	14.8 ± 10.7	17.0 ± 13.1	15.1 ± 10.5
FAILED TO LEARN c	N(%)	1(4.2)	0(0.0)	1(5.0)	0(0.0)
SESSION 2a	N	23	25	19	24
TRIALS TO CRITERION	MEAN±S.D.	6.0 ± 1.7	6.8 ± 2.7	6.5 ± 2.5	6.7 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.09 ± 0.14	0.13 ± 0.19	0.12 ± 0.24	0.12 ± 0.13
LATENCY TRIAL 1b	MEAN±S.D.	11.0 ± 6.1	10.9 ± 8.6	9.5 ± 4.6	12.0 ± 7.2

a. Session 1 (Learning Phase) and Session 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in session 1 of testing (learning); session 2 (retention) values for these rats were excluded from group averages and statistical analyses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

F_1 C-sectioning and Litter observations: The C-section delivery observations such as corpora lutea, implantations, litter sizes, and percent male fetuses were comparable among the four maternal dose groups and did not differ significantly. However, the number of dams with any resorptions increased dose dependently. The percent of dams with any resorptions were 20, 36, and 54 in the 5, 10, and 20 mg/kg/day.

F_1 C-sectioning and Litter observations:

Parameter	Dose (mg/kg/day)			
	0	5	10	20
F_1 Postnatal Parameters/ Reproductive Maturity/ Fertility Parameters				
Dams w/any resorptions N (%)	9 (41%)	5 (20%)	9 (36%)	13 (54%)

F_2 findings: The gross evaluations of the F_2 generation were based on the observation of the external alterations and fetal body weights. No fetal gross alterations were identified in the F_2 fetuses. The fetal body weights were comparable in the four maternal dose groups.

2.6.6.6 Local tolerance

Study Title: *In Vitro* Hemolysis Study (Modified ASTM – Direct Contact Method): AQUAVAN® Injection 35 mg/mL

Key study findings

- The test article was incubated with the diluted rabbit blood to evaluate its hemolytic potential.
- The test article was hemolytic (91%) under this experimental condition. The test article was formulated in water which might have result in hemolysis. As this is not the proposed clinical formulation, the results of the study are not relevant to the safety of the proposed drug product.

Study number: 03T-22169-01
Volume # and page #: Module 4-eCTD submission; Page#: 1-8
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 11-23-03
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715 (35 mg/mL), 176I0603, purity not mentioned

Formulation/vehicle: Water
Negative control: High density polyethylene
Positive control: Water

Study design: The study was designed to assess the compatibility of the test article with blood. Diluted rabbit (n=3) blood was added either to the test article directly, negative control, or positive control. The hemolysis caused by the direct contact of blood with the undiluted test article was measured.

Results: Under this experimental condition the test article was severely hemolytic.

Study title: Peri-vascular Irritation Study in the Rabbit

Key study findings

- The local tissue response a single dose (0.1 mL) of the test article, saline (negative control), and propofol were injected perivascularly into separate sites along the dorsal, ventral, and lateral veins of both ears in rabbits.
- The test article, GPI 15715 did not induce perivascular irritation at Day 3 and Day 11 after a single administration of 0.1 mL (200 µg) around the ear vein in rabbits.

Study number: Loc-tol\3000-15715-00-09g
Volume # and page #: Module 4-eCTD submission; Page#: 1-18
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 09-11-2000
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: Two compounds GPI 15715 and propofol were tested
GPI 15715, 217-8-21-3, 84.8%
Propofol, 00E311
Formulation/vehicle: Saline (0.9%)
Drug, lot #, and % purity: 21708-21-3

Study design: To evaluate the local tissue response a single dose (0.1 mL) of the test article, saline (negative control), and propofol were injected perivascularly into separate sites along the dorsal, ventral, and lateral veins of both ears in rabbits (n=6). The injection sites were observed daily for erythema and edema and scored according to a subjective rating. The macro and microscopic evaluation were also conducted at Day 3 and Day11 from three rabbits in each day.

Results: Under the condition of the study, the test article GPI 15715 did not produce any irritation in rabbit. In rabbits, propofol by itself induced histopathological changes associated with necrosis at Day 3 no such changes were noted at Day 11. However, there was a complete recovery at Day 11 which according to the study conductor was due to the small amount of the material injected.

Study title: *In Vitro* Hemolysis Study (Modified ASTM – Direct Contact Method): GPI 15715

Key study findings

- The hemolytic potential of the test article formulated in saline was incubated with the rabbit (n=3) blood to evaluate its hemolytic potential.
- Under this experimental condition the test article exhibited a maximum hemolytic index of 1.6% and thus considered not hemolytic. The positive control showed a hemolytic index of 11.9%.

Study number: Loc-tol\3000\15715-00-10g
Volume # and page #: Module 4-eCTD submission; Page#: 1-9
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 09-11-2000
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 17515, 217-8-21-3, 84.8%
Formulation/vehicle: Saline
Negative control: High density polyethylene
Positive control: Not mentioned

Study design: The study was designed to assess the compatibility of the test article with blood. Diluted rabbit (n=3) blood was added either to the test article (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL, negative control, or positive control. The hemolysis caused by the direct contact of blood with the test article was measured.

Results: Under this experimental condition the test article exhibited a maximum hemolytic index of 1.6% and thus considered not hemolytic. The positive control showed a hemolytic index of 11.9%.

Study title: Hemolysis Test (ASTM Method) Direct Contact Method

Key study findings

- To evaluate the hemolytic potential, rabbit blood was incubated either to the different concentrations of GPI 15715 formulated in phosphate buffered saline, negative control, or comparator.
- The test article demonstrated a maximum hemolytic index of 1.6% in this study.

Study number: Loc-tol\22737
Volume # and page #: Module 4-eCTD submission; Page#: 1-2
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 08-05-2004

GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715, 176I0603, purity not provided
Formulation/vehicle: Sterile Phosphate buffered in saline (PBS)
Negative control: PBS
Positive control: Positive control data provided, but the name of the compound used as positive control was not provided.

Study design: The study was designed to assess the compatibility of the test article with blood. In this method citrated rabbit (n=3) blood was added either to the different concentrations of the test article (1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL), negative control, or comparator. The blood was diluted to 10 mg/mL prior to the mixing with the test article to achieve a concentration of the plasma free hemoglobin approximately 2 mg/mL. The hemolysis caused by the direct contact of blood with the test article was measured.

Results: The test article demonstrated a maximum hemolytic index of 1.6% in this study.

Study 22738: Hemolysis Test (ASTM Method) Direct Contact Method

Key study findings

- The hemolytic potential of GPI 151715 (35 mg/mL stock solution was assessed by incubating it at different concentration with rabbit blood in an ex vivo assay.
- Under this experimental condition the hemolytic index of the test article was determined to be 3.1% and considered slightly hemolytic.

Study number: Loc-tol\22738 b(4)
Volume # and page #: Module 4-eCTD submission; Page#: 1-2
Conducting laboratory and location: [redacted]
Date of study initiation: 08-09-2004
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: GPI 15715 (35 mg/mL); drug lot# and purity, not provided
Formulation/vehicle: Sterile Phosphate buffered in saline (PBS)
Negative control: PBS
Positive control: Positive control data provided, but the name of the compound used as positive control was not provided

Study Design: The study was design to asses the hemolytic potential of GPI 15715 (35 mg/mL stock solution). Citrated rabbit (n=3) blood was diluted with PBS to ensure a concentration of the plasma free hemoglobin of 2 mg/mL. The test articles of different concentrations (0.1, 0.4, and 1.0 mg/mL) were then mixed with the diluted blood and the hemolytic potential was measured by spectrophotmetric analysis.

Results: Under this experimental condition the hemolytic index of the test article was determined to be 3.1% and considered slightly hemolytic. The negative control value was 0.2% and the positive control value was 11.4%.

Study title: Hemolysis Test (ASTM Method) Direct Contact Method

Key study findings

- In an ex vivo assay, rabbit blood was incubated with different concentrations of the marketed drug Diprivan, a lipid formulation of propofol (10 mg/mL).
- Diprivan demonstrated a hemolytic index of 25.8% and 4.8% at 0.05 and 0.005% concentration respectively in this study.

Study number: Loc-tol\22739
Volume # and page #: Module 4-eCTD submission; Page#: 1-8
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 08-06-2004
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: Diprivan (10 mg/mL); Lot# and purity not provided.
Formulation/vehicle: Sterile Phosphate buffered in saline (PBS)
Negative control: PBS
Positive control: Positive control data provided, but the name of the compound used as positive control was not provided.

Study design: The study was designed to assess the compatibility of the test article with blood. In this method citrated rabbit (n=3) blood was added either to the different concentrations of the test article (0.05 and 0.005 mg/mL), negative control, or comparator. The blood was diluted to 10 mg/mL prior to the mixing with the test article to achieve a concentration of the plasma free hemoglobin approximately 2 mg/mL. The hemolysis caused by the direct contact of blood with the test article was measured.

Results: The test article demonstrated a hemolytic index of 25.8% and 4.8% at 0.05% and 0.005% concentration respectively in this study.

Study title: Single Dose Toxicity/Irritation Study with GPI 15715 by Subcutaneous Dosing in Sprague-Dawley Rats

Key study findings

- GPI 15715 was administered subcutaneously (0, 10, 35, 45, 60, 100, 150, and 200 mg/kg) in the right flank of the Sprague Dawley rats (3/sex/group) at Day 1 of the study; clinical observations, body weights, gross lesions, and histopathology was

evaluated at necropsy on Day 2. The left flank of the rats was injected with water for controlling the experimental procedure.

- In doses ≥60 mg/kg increased incidence of subcutaneous inflammation with minimal-slightly severe in nature was observed in all animals in the right flank. Myositis of the panniculus carnosus was also noted. The left flank did not show any inflammation. Based on these observations a NOAEL of 45 mg/kg was **established (Sponsor's NOAEL= 100 mg/kg).**

Study number: Loc-tol\GPI 15715-tox-04-026
Volume # and page #: Module 4-eCTD submission; Page#: 1-103
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 05-18-2004
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, 142506A 98.6%, 142506B 99.6%
Formulation/vehicle: Water

Study design: The test article was administered subcutaneously (0, 10, 35, 45, 60, 100, 150, and 200 mg/kg) in the right flank of the Sprague Dawley rats (3/sex/group) at Day 1 of the study; clinical observations, body weights, gross lesions, and histopathology was evaluated at necropsy on Day 2. The left flank of the rats was injected with water for controlling the experimental procedure.

Results: The clinical observations associated with slight uncoordinated movement were noted in all animals at 35 mg/kg dose. In doses ≥45 mg/kg lethargy, flat posture, sedation, and uncoordinated movements were noted at Day 1 up to 4 hrs post administration of the test article. In doses ≥60 mg/kg increased incidence of subcutaneous inflammation with minimal-slightly severe in nature was observed in all animals in the right flank. Myositis of the panniculus carnosus was also noted. The left flank did not show any inflammation. Based on these observations a NOAEL of 45 **mg/kg was established (Sponsor's NOAEL= 100 mg/kg).**

Study title: Single Dose Vascular Irritation Study of GPI 15715 (Aquavan®) in Rabbits

Key study findings

- The potential vascular irritancy of GPI 15715 was determined by a single intravenous administration of the test article (0.1 mL) in the right ears of the New Zealand white rabbits (n=7). In the left ear vehicle was administered IV as controls.
- Vascular wall irritancy with mild to moderate erythema and edema were noted in all rabbit under this experimental condition in both ears. The incidence and

severity decreased with the progression of the study. Because vascular irritancy was comparable in control treated ears, the findings are believed to be not test article related.

Study number: Loc-tol\gpi-15715-tox-04-007
Volume # and page #: Module 4-eCTD submission; Page#: 1-58
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 12-01-2004
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 17515 (35 mg/mL), 176I0603, 98.9%
Vehicle: Aquavan vehicle, 11862-42, 100%
Note that the test article and the control were received by the CRO as ready to use, no formulation was necessary.

Study design: The potential vascular irritancy of GPI 15715 was determined by a single intravenous administration of the test article (0.1 mL) in the right ears of the New Zealand white rabbits (n=7). In the left ear vehicle were administered IV as controls. All rabbits were terminated one week following dose administration. The vascular irritancy was analyzed based on Draize scale; local histopathology analyses were also conducted.

Results: Vascular wall irritancy with mild to moderate erythema and edema were noted in all rabbit under this experimental condition in both ears. The incidence and severity decreased with the progression of the study. Because vascular irritancy was comparable in control treated ears, the findings are believed to be not test article related.

Study title: A Primary Skin Irritation Study in Rabbits with GPI 15715 (FOSPROPOFOL®)

Key study findings

- The potential for the irritation and the corrosive effect, the test article was examined after its application to the skin of six New Zealand white rabbits. The test sites were observed 1, 24, 48, and 72 hrs post dosing for irritancy and scored and analyzed according to the Draize scale.
- The test article is considered non irritant under this experimental condition.

Study number: Loc-tol\gpi-15715-tox-05-032
Volume # and page #: Module 4-eCTD submission; Page#: 1-34
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 05-19-2005
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % potency: GPI 15715 (35 mg/mL), 900337, 101%

Negative control: 0.9% saline
Note that the test article was received by the CRO as ready to use, no formulation was necessary.

Study design: To evaluate the potential for the irritation and the corrosive effect, the test article was applied to the skin **of six New Zealand white rabbits (0.5 mL in a 1'x1' gauze** patch). The patch was applied in two skin sites/animal, in one area the hair was clipped in the other area, the hair was clipped and the skin was abraded carefully and superficial so that the drug can cross the stratum corneum but the dermis is not damaged (that is no bleeding occurred). The test sites were observed 1, 24, 48, and 72 hrs post dosing for irritancy and scored and analyzed according to the Draize scale.

Results: The intact skin showed slight erythema 1/6 animals at one hr post dosing which resolved at 24 hrs post dosing. The abraded skin showed slight irritancy 1/6 animals at one hr post dosing which resolved at 48 hrs post dosing. The primary irritancy index (PII) was observed to be 0.08% in these animals. According to the dermal evaluation scale $\geq$5.0 is considered to be a primary irritant. Therefore, the test article is considered a non irritant under this experimental condition.

Study title: A Primary Eye Irritation Study in Rabbits with GPI 15715 (FOSPROPOFOL®)

Key study findings

- The ocular irritancy was assessed by intraocular instillation of the test article (0.1 mL) in the conjunctival space of the New Zealand white rabbits. The potential for the ocular irritancy and necrosis were analyzed macroscopically and microscopically 72 hrs post dosing.
- The test article did not cause any ocular irritancy under this experimental condition.

Study number: Loc-tol\gpi-15715-tox-05-031
Volume # and page #: Module 4-eCTD submission; Page#: 1-31
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 05-19-2005
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % potency: GPI 15715 (35 mg/mL), 900337, 101%
Negative control: 0.9% saline
Note that the test article was received by the CRO as ready to use, no formulation was necessary.

Study design: The ocular irritancy of the New Zealand white rabbits were assessed by intraocular instillation of the test article (0.1 mL/3.5 mg/mL) in the conjunctival space.

The potential for the ocular irritancy and necrosis were analyzed macroscopically and microscopically 72 hrs post dosing.

Results: The test article did not cause any ocular irritancy under this experimental condition.

2.6.6.8 Special toxicology studies

Study title: A Dermal Sensitization Study in Guinea Pigs with GPI 15715 (AQUAVAN®): Standard Buehler Design

Key study findings

- GPI 15715 was tested for dermal sensitization using Buehler design in guinea pigs.
- No contact sensitization was noted after challenging the animals with the test article indicating that the test article is negative for dermal sensitization under this experimental condition.

Study number: Other-tox-stud\42371-antigen\gpi-15715-tox-05-033
Volume # and page #: Module 4-eCTD submission; Page#: 1-54
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 05-26-05
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % potency: GPI 15715 (35 mg/mL), 900337, 101%
Positive control: 1-chloro-2, 4-dinitrobenzene (DNCB)

Methods:

Doses: Topical, 100% of GPI 15715 (35 mg/mL), administered 3x/week for 3 weeks (9-induction exposure in total).

Study design: To evaluate the dermal sensitization potential of the test article, Hartley guinea pigs (10/ sex/group) were induced either with the test article or with the positive control (0.1% w/v DNCB in acetone/ethanol) by dermal exposure for a 3 week time period. Following a 2-week rest period the animals were challenged with the test article or different concentrations of the positive control. The challenge response in the test article treated animals was compared with those of the challenged control animals (naïve animals which were not exposed to induction). The protocol followed the standard Buehler test for the assessment of the hypersensitivity reaction. Following is the **Sponsor's study design.**

Study design table:

Group	No. of Animals	Phase/Treatment	
		Induction 1 - 9	Challenge
Test	20	Test Article	Test Article
Challenge Control	10	--	Test Article
DNCB Test	10	DNCB (0.1%)	DNCB (0.1% and 0.05%)
DNCB Control	10	--	DNCB (0.1% and 0.05%)

Results:

GPI 15715 produced minimal dermal reaction following induction. No contact sensitization was noted after challenging the animals with the test article indicating that the test article does not have a potential for dermal sensitization under this experimental condition. The positive control, DNCB produced hypersensitivity reaction suggesting the validity of the assay.

Study title: 7-Day Oral Toxicity Study with GPI 15715 by Daily Gavage in Sprague Dawley Rats

Key study findings

- GPI 15715 was administered in rats (5/sex/group) orally at different dosages (0, 4, 20, 50, and 100 mg/kg) for seven consecutive days and standard toxicity parameters such as clinical observations, body weight, food consumption, clinical pathology, and histology were assessed.
- NOAEL was determined to be 100 mg/kg since no toxicity was observed. The study indicates that GPI 15715 was not active when administered orally under this experimental condition.

Study number: Other-tox-stud\42371-antigen\gpi-15715-tox-05-033
Volume # and page #: Module 4-eCTD submission; Page#: 1-8

Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 05-18-2004
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: GPI 15715, 142506A 98.6%, 142506B 99.6%
Formulation/vehicle: Water

Methods:

Doses & Study design: The test article was administered by oral gavage (0, 4, 20, 50, and 100 mg/kg) to Sprague Dawley rats (5/sex/group) for seven consecutive days. The animals were sacrificed after 24 hrs post last dosing. The parameters studied include

clinical observations, body weights, gross lesions, and histopathology of the gastrointestinal tract.

Results:

The clinical observations associated with uncoordinated movement and flat posture were noted in all high dose females at Day 2 and 4 of the treatment. No other treatment related changes were noted. Therefore, the NOAEL for GPI 15715 after oral gavage administration was established to be 100 mg/kg.

2.6.6.9 Discussion and Conclusions

GPI 15715 is a prodrug of propofol which after enzymatic digestion with alkaline phosphatase in vivo is metabolized to propofol, formic acid, and phosphate. The toxicity studies were designed to compare the toxicity derived from GPI 15715 and propofol. The toxicity evaluation of the test article with sedative properties was conducted by applying unique study designs. The toxicity was evaluated in most case by a combination of a single bolus and continuous infusion to mimic the therapeutic indication to mimic the clinical scenario. The toxicity study design to mimic the clinical application for anesthetics are challenging in general as the animals are mostly sedated which might result in indirect toxicity resulting from less food consumption, muscle weaknesses etc. Also, the total daily intakes to achieve frank toxicity were obtained either by increasing the infusion time using the same dose or by differing the dose with a constant infusion time. The active metabolite propofol is an FDA approved marketted drug for anesthesia. Therefore, the safety assessment of GPI 15715 was based on the strategy to evaluate any toxicity related to GPI 15715 which might result from the formation of the formate and the phosphates. The general toxicity of GPI 15715 was thus not only evaluated in the single and repeat dose toxicity studies in rodents and non rodents but the resulting toxicity was compared either with propofol or formaldehyde directly.

During the drug development process of GPI 15715, the major toxicity issues were formation of the formaldehyde as a metabolite which is a known genotoxic carcinogen, and ocular toxicant. The initial clinical development program was put on hold by the Agency until the Sponsor explained and rationalized the toxicity related to formate production. The Sponsor provided the data that in presence of formaldehyde dehydrogenase which is present in the body, the test article is not genotoxic. In the EOP II and the preNDA meeting, the Sponsor was asked by the Agency to address the issue with the formate related ocular toxicity if any. In most of the pivotal toxicity studies, the Sponsor compared the toxicity of the prodrug with its active metabolite propofol. The doses were chosen for animals to received theoretically similar quantity of propofol. In a few cases, a satellite group of animal was administered formaldehyde that would result in a theoretically higher exposure of formate production in the body to evaluate any toxicity that might result from the formate production in the body after the GPI 15715 administration. The plasma formate concentrations after GPI 15715 administrations in all of the toxicokinetics studies conducted under this submission were comparable to the

background. There was no test article related ocular toxicity in any of the animal species studied.

The summary of toxicity findings following GPI 15715 administrations from rat, dog, and cynomolgus monkeys are tabulated below. The toxicity findings from the GPI 15715, propofol, formaldehyde administrations were also compared in the following table.

GPI 15715: Summary of Toxicity Findings in Rat, Dog, and Monkey from Repeat Dose Toxicity Studies:

Study/ Number of animals/ Dose	Exposure/AUC (µg.h/mL) & Cmax (µg/mL)			Major Findings
	GPI 15715	GPI 15715 derived Propofol	Formate	
Monkey 1-month TDI= 165 mg/kg/day, HED= 53 mg/kg/day	91.5 46	23.8 5.5	786 34.8/ same as control	Lymphoid cell aggregates in lungs (associated w/inflammation), heart, skeletal muscle eye, and lacrimal gland Congestion in kidney; Parasitic cyst in GI tract; Skin disorders such as squamous cell hyperplasia, hypertricohsis, hemorrhage;
Dog 14-day TDI= 133 mg/kg/day HED= 74 mg/kg/day	95.7 23	24 20	ND	Chronic inflammation in lungs; Thickening of the skin in the injection site; Bone marrow cell hyperplasia; Metaplasia in trachea;
Rat 14-day 1, 2, 4 hr infusion /day TDI= 190 mg/kg/day HED= 30.6 mg/kg/day	74 46	21 9	ND	Chronic inflammation in lungs; Acute inflammation in liver; Bone marrow cell hyperplasia Cardiomyopathy@ mid dose, Congestion in kidney Chronic active inflammation in the injection site; Spleen extramedullary hematopoesis;

The test article was compared with propofol for understanding its toxicity profile in most of the toxicity studies. The major toxicity findings were decrease in erythrocyte parameters, increase in triglycerides, acidosis, and histopathological lesions in lungs, heart, kidney, liver, trachea, spleen, skin, skeletal muscle, bone marrow hyperplasia, and GI tract.

GPI 15715 induced hematological changes include decrease in hemoglobin, hematocrit, and RBC in all of the different species studied 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. These changes might be related to the bone marrow cell hyperplasia also. Similar effects on the erythrocytic parameters are reported with propofol administration. In most of the toxicity studies conducted with GPI 15715, the clinical pathology changes include changes in the liver enzymes such as alkaline phosphatase which is associated with the metabolism of the test article metabolism. The clinical pathological changes also include a significant increase in the triglycerides level which the Sponsor attributes to changes in the corticosteroid levels. Although not evaluated with GPI 15715 toxicity studies, propofol is known to increase plasma corticosterone level.

According to the Sponsor, the immunosuppressive effects observed after long term treatment with GPI 15715 as noted by increase parasitic cyst in gut, and bacterial infection in skin in the monkey model might be attributed to the plausible increase corticosteroids secretion as evidence indirectly by increased triglycerides formation. **However, the Sponsor's hypothesis** is not supported by any data.

The cardiovascular effects manifested as changes in the heart rate and mean arterial pressure is consistently noted in all test articled treated animals. The test article treated animals in most cases either had inflammation in lungs or increase edema/effusion in lungs which is consistent with cardiac insufficiency which might be related to the exaggerated pharmacology of the test articles. In a 48-h, continuous-infusion monkey study in which animals received either GPI 15715 or propofol distinctive microscopic myocardial changes, including subendocardial degeneration, neutrophilic infiltrates, and karyomegaly were noted. GPI 15715 administered by intermittent infusion of up to 4 weeks demonstrated neither significant changes in ECG patterns nor cardiotoxicity in the monkey. Skeletal myodegeneration was also observed accompanied by edema, hemorrhage, acute inflammation, and lymphocytes infiltration. Skeletal and cardiac muscle pathology has been reported in patients receiving higher than typical rates of propofol infusion (Stelow et al., 2000). This indicates that the skeletal muscle degeneration in the monkeys might have human relevance and this effect is related to the propofol exposure from GPI administration.

There was one unique finding in stomach of one GPI 15715 treated dog. The lesion in the stomach was associated with hemorrhage and necrosis consistent with histamine release, although histamine release was not measured in the toxicity studies using GPI 15715, propofol is reported to have histamine release effect in previous toxicity studies. Therefore, the observation of the stomach lesion might be related to the histamine release after GPI 15715. The metaplasia in the tracheal area was noted in all species. The lesions in trachea is believed to be associated with the cannulation, however, in some instances the severity indexes were observed to be higher in the test article treated animals compared to the saline controls. The biomaterial content of the cannulation was not compatible to the animals.

Comparison of Toxicity Findings w/GPI 15715 in Rat, Dog, and Monkey to Propofol:

Study/ Number of animals/ Dose	Exposure/AUC (µg.h/mL) & Cmax µg/mL)		Major Findings
	Propofol	Formate	
Dog 3/sex/group 14-day TDI= 51 mg/kg/day HED= 28 mg/kg/day	20 14	ND	Chronic inflammation in lungs in interstitium, higher in incidence w/propofol than GPI 15715 treated animals; Chronic inflammation in lungs in visceral pleural area, higher in incidence than GPI 15715 treated animals; Thickening of the skin in the injection site, lesser in incidence than GPI 15715 treated animals; Bone marrow cell hyperplasia, similar to GPI 15715 treated animals; Metaplasia in trachea, similar to GPI 15715 treated animals;
Rat 14-day 5/sex/group repeat dose, 4-hr/day / continuous infusion TDI= 80 mg/kg/day HED= 12.9 mg/kg/day	6.8 1.7	ND	Chronic inflammation in lungs, similar to GPI 15715 treated animals; Acute inflammation in liver, similar to GPI 15715 treated animals; Bone marrow cell hyperplasia, similar to GPI 15715 treated animals; Cardiomyopathy@ mid dose, similar to GPI 15715 treated animals; Congestion in kidney, similar to GPI 15715 treated animals; Chronic active inflammation in the injection site, lesser in incidence than GPI 15715 treated animals; Spleen extramedullary hematopoesis, similar to GPI 15715 treated animals;
Monkey 3/sex/group 24-48 hrs/ continuous infusion Male: TDI= 730 mg/kg/day; HED= 235 mg/kg/day	187 18	495 29 Same as back -ground	Myocardial degeneration w/neutrophilic infiltration, and karyomegaly observed in one animal, similar to GPI 15715 treated animals; Skeletal muscle myofibers w/perimysium, myofibers degeneration/regeneration, similar to GPI 15715 treated animals; Spleen lymphcytosis, similar to GPI 15715 treated animals; No skin findings

The toxicity profile of GPI 15715 was also compared to that of formaldehyde and described in the following table. Note all of the toxicities observed in the skin following GPI 15715 were similar to formaldehyde treated animals.

Comparison of Toxicity Findings w/GPI 15715 in Rat, Dog, and Monkey to Formaldehyde

Study/ Number of animals/ Dose	Exposure/ Formate AUC µg.h/mL Cmax µg/mL	Major Findings
Monkey	855	No lymphoid cell aggregates, unlike GPI 15751 treated

1-month TDI= 65.7 mg/kg/day HED= 21 mg/kg/day	**70.4**	**animals; Parasitic cyst in GI tract, lesser in incidence than GPI 15751 treated animals; Skin disorders such as squamous cell hyperplasia, hyperkeratosis, hypertricohsis, hemorrhage; like GPI 15751 treated animals;**

GPI 15715 was also examined in the local tolerance and special toxicity studies in the single dose ocular safety, skin irritation, hemolytic activity, hypersensitivity, and subcutaneous toxicity study and a 7-day repeat dose oral toxicity study. The test article was found to be hemolytic only in the non physiological concentration. Skin irritation was observed in rat after subcutaneous administration of the compound at a dose > 100 mg/kg (HED=16 mg/kg). No ocular irritation was noted in rabbit after the local administration of the test article (3.8 mg/kg, HED=1.1 mg/kg). GPI 15715 was not hypersensitive as indicated from the Buehler test in guinea pig. Local irritation of skin was not observed after topical administration with the test article after a single administration.

GPI 15715 was evaluated in a complete battery of the reproductive toxicity studies by bolus intravenous administration. The study protocols followed the ICH S5 Guidance documents, all of the studies are considered valid because maternal toxicity was observed in the maximum tolerated dose.

The major findings from the reproductive toxicity studies were test article related increase resorptions of the fetuses, malformation in rabbit, 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 GPI 15715 are related to propofol. The skeletal malformations and variations observed in the rabbits were, however, not reported in propofol reproductive toxicity studies GPI 15715 tissue distribution study did not include whole body autoradiography, olfactory area was not counted for GPI 15715 related radioactivity, therefore it is not known whether there might be direct correlation between the skeletal tissue anomalies in the nasal area with GPI 15715 administration. GPI 15715 induced acidosis, however, was noted in almost all the toxicity studies conducted. Acidosis is well known to induce skeletal anomalies including incomplete ossification. Thus the test article related changes in the embryofetal development might be related to the secondary pharmacodynamics effect of the test article. Regardless of the mechanism mediating these changes, it is recommended that GPI 15751 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 clinically relevant. However, under the current ICH guidelines, the reproductive toxicity studies are valid and did produce reproductive toxicity with GPI 15715 administration which in reviewer's opinion should be reported

b(4)

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The current therapeutic indication of fospropofol disodium is single dose short term sedation [redacted] 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 clinics is very narrow. However, for short term, single dose administration no acute toxicity is predicted from the non clinical safety evaluation. b(4)

The general toxicity findings related to skin lesion 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.

The reproductive and developmental toxicity findings include resorptions of fetus, malformations, and skeletal variations in rats and rabbits. The reproductive toxicity assessments were 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 and not predicted. [redacted] 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)

Unresolved toxicology issues (if any): Skin lesions with GPI 15715 are unique finding and may be related to the formaldehyde production. Bacterial infection of the skin lesion was noted in one study which might indicate immune suppression.

Recommendations:

The non clinical studies to understand the mechanism of the formation of the skin lesions is recommended for the prolonged administration of the test article.

The fospropofol sodium is recommended to be examined in the juvenile studies, prior to its approval in the pediatric population.

b(4)

[redacted]

APPENDIX/ATTACHMENTS

Reference List

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