CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205579Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA: 205579 SDN1, SDN27	Submission Date(s): 1/22/2014, 6/20/2014
Brand Name	Ryanodex
Generic Name	Dantrolene Sodium Lyophilized Suspension for Injection
Clinical Pharmacology Reviewer	Srikanth C. Nallani, Ph.D.
Team Leader	Yun Xu, Ph.D.
OCP Division	Division of Clinical Pharmacology II
OND Division	Anesthesia, Analgesia and Addiction Products
Sponsor	Eagle Pharmaceuticals Inc.
Relevant IND(s)	105411
Submission Type; Code	Original Application; 505(b)(2)
Formulation; Strength(s)	Lyophilized Suspension for Injection; 250 mg/vial, 50 mg/mL after reconstitution
Indication	Prevention and (b) (4) of Malignant Hyperthermia
Proposed Dosage Regimen	IV bolus at 1 mg/kg for (b) (4) 2.5 mg/kg bolus starting approximately 1.25 hours before anticipated anesthesia.

Table of Contents

1 Executive Summary

1.1 Recommendation

The submission is acceptable from a Clinical Pharmacology perspective provided that a mutually satisfactory agreement can be reached between the sponsor and the Agency regarding the language in the package insert.

1.2 Phase IV Commitments

None

1.3 Summary of Clinical Pharmacology Findings

Eagle Pharmaceuticals Inc., submitted NDA 205579 to market Dantrolene Sodium Suspension (250 mg/ 5mL vial) for the prophylaxis and treatment of Malignant Hyperthermia under a 505(b)(2) pathway using NDA 018264 ((Dantrium IV) as the listed drug. Malignant Hyperthermia (MH) is an orphan disease and the sponsor obtained an orphan designation for the product (Designation number 03-1797). Previously approved Dantrium Infusion (IV) was used to treat MH with limitations on getting adequate therapeutic benefit. To support this 505(b)(2) NDA, sponsor conducted a single clinical study "EGL-Dantrolene-1201" to generate the safety database and clinical experience for Ryanodex Suspension for Injection. The sponsor evaluated PK of Dantrolene sodium suspension (Ryanodex) and compared its safety, tolerability and pharmacokinetics to Dantrolene Sodium (solution) for Injection (Dantrium IV) in healthy volunteers.

Dantrium Intravenous (dantrolene sodium for injection) which contains 20 mg of dantrolene sodium per vial is the currently marketed product for the treatment of malignant hyperthermia (MH). The labeled dose of dantrolene sodium for an MH crisis ranges from 1 mg/kg to 10 mg/kg. Therefore, the dose for a 70 kg patient can reach 700 mg. This dose would require a total of 35 vials to be reconstituted immediately, each with 60 mL of WFI with a total volume to be rapidly administered exceeding 2 liters.

The sponsor contends that a nano-particle suspension offers a high concentration for delivery, coupled with the opportunity for rapid dissolution of the particles upon dilution in the blood due to their large surface area. A product presentation of 250 mg dantrolene sodium per vial to be reconstituted with 5 mL of water for injection was chosen to accommodate the dose range of 1 to 10 mg/kg. This presentation would require less than 3 vials and less than 15 mL to deliver a dose of 700 mg.

Ryanodex is supplied as a sterile lyophilized powder containing 250 mg of dantrolene sodium (b) (4) 125 mg of mannitol, 4 mg of povidone (b) (4) and 25 mg of polysorbate 80 per vial, hydrochloric acid and sodium hydroxide.

Ryanodex (after reconstitution with 5 mL sterile water for injection) is a nanoparticle suspension designed for rapid intravenous administration. Dissolution of the nanoparticle formulation was evaluated in an in vitro study utilizing human plasma. Biopharm reviewer Dr. John Duan agreed with the sponsor that there were no visible solid material present when the dissolution was evaluated in human plasma. Most of the suspension dissolved in human plasma within a minute followed by complete dissolution as evaluated in the in vitro dissolution study. Additionally, the human plasma samples in

the dissolution study had slight orange to yellow color, particularly at high concentrations. The drug product is orange to yellow in color and the preclinical studies indicate yellow color stain in tissues after significantly high dosing with dantrolene in animal toxicology studies. In addition, the effect of Ryanodex or Dantrium on hemolytic potential was evaluated in whole blood incubations and found not to be an issue (See Dr. Jay Chang's Pharmacology Toxicology review).

Clinical PK study "EGL-Dantrolene-1201" was initiated with (b) (4) See attached synopsis) and there after due to logistical considerations the sponsor hired Comprehensive Clinical Development (Tacoma, WA) to finish the study.

Part 1 was a dose escalation design where each dose group received Ryanodex at doses of 1.0 (n=4), 1.75 (n=4) or 2.0 mg/kg (n=2) via 30-second bolus injection, except for 4 subjects in Cohort 3b ('Special Cohort' 1.75 mg/kg Ryanodex as a 30 sec bolus vs. n=4 as a 5-minute infusion). A total of 19 subjects received Ryanodex, 4 subjects received placebo. Pharmacokinetic samples were drawn over the first 72 hours. Subjects who received study drug and had an adequate number of concentration-time points to delineate a PK profile were included in the PK population for analysis. PK sample analysis for Part 1 was conducted by (b) (4) Part 1 of the study was partially evaluated by (b) (4) with placebo as a control, however after the initial Ryanodex dose escalations it became apparent that the planned study design was not adequate to generate useful data to determine the safety and tolerability of Ryanodex relative to Dantrium. Results of (b) (4) conducted part 1 of the clinical study are described in attached section (4.2.4).

Therefore, the study was contracted to CCD to complete the dose escalation cohorts and the comparison of safety Ryanodex vs. Dantrium at different dose levels. The sponsor amended the protocol to add safety monitoring measures. Additionally, the study was modified as follows:

- Part 1 was a dose escalation design where each treatment group received either Ryanodex or Dantrium at doses of 1.0, 1.75, 2.0, 2.25 or 2.5 mg/kg.
- Part 2 was conducted as a randomized, two-way crossover; subjects received 2.5 mg/kg of Ryanodex or Dantrium. Doses of 1.0 to 2.25 mg/kg were administered to male subjects (only) and the dose of 2.5 mg/kg was administered to both male and female subjects.

All Ryanodex doses were administered as a 60 second continuous IV push, and all Dantrium doses were administered as a 50 mL/min infusion, corresponding to 16.5 mg/min dantrolene sodium (duration of Dantrium dose administration varied by dose group and by subject body weight; range of dose duration was approximately 4 to 13 mins).

Details of Treatment Administration

Two peripheral, contralateral IV lines were started and maintained in each of the subjects. The IV line in one arm was used for study drug administration, and served as emergency venous access after dosing was completed.PK blood sampling was drawn from the IV line in the opposite arm, and served as backup for emergency venous access if required. Both

lines were infused with saline sufficient to maintain patency after dosing and between blood sampling.

An 18g IV catheter was inserted into each participant's forearm or antecubital area for dosing. A small bore extension tubing set was attached to the IV catheter. At the end of the small bore extension tubing, a 3-way stopcock was engaged. The subject was supine in a recliner chair with a privacy curtain draped over the dosing area (arm).

Per convention for both Dantrium and Ryanodex, the "dose time" of reference for all post-dose procedures was the time relative to the end of dose administration. The infusion start time for Ryanodex was 1 minute prior to the dose time. The Dantrium infusion start time varied for each subject depending on the subject's weight and dose cohort.

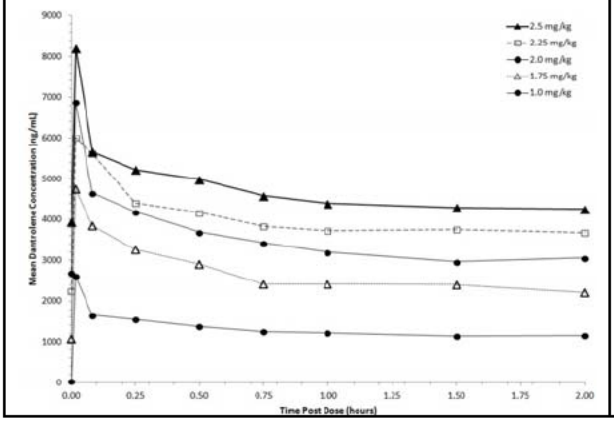
Pharmacokinetic samples were taken over a period of 72 hours post-dose, with PK samples over the first 2 hours taken at; predose, immediately post-dose, and at 1, 5, 15, 30, 45, 60, 90 and 120 minutes following the end of dose administration. The pharmacokinetic (plasma) sample analysis was performed by (b) (4) Bioanalytical methodology was similar between (b) (4) employed PK analysis (See Section for bioanalytical validation 4.2.1).

Part 1 PK: Dantrolene PK following Ryanodex and Dantrium administration

Dose proportionality for dantrolene and 5-hydroxydantrolene within a treatment group was evaluated using Cmax and AUC0-inf following drug administration. Dose proportionality is evident for both Ryanodex and Dantrium at doses of 1.0 mg/kg to 2.5 mg/kg for dantrolene Cmax and 5-hydroxydantrolene Cmax and AUC0-inf.

Mean Dantrolene Plasma Concentration Vs. Time by Dose Group (0- 2 hrs) following Ryanodex (Left) or Dantrium (Right) administration.

Ryanodex



Dantrium

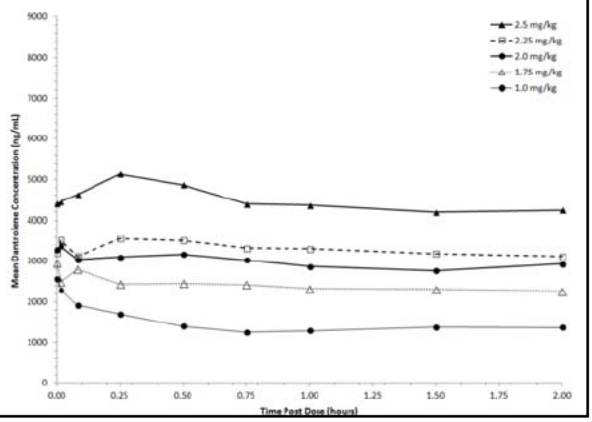


Table: Summary of Dantrolene Plasma PK Parameters following 1 mg/kg, 1.75 mg/kg, 2 mg/kg, 2.25 mg/kg of Treatment A (Ryanodex) and Treatment B (Dantrium).

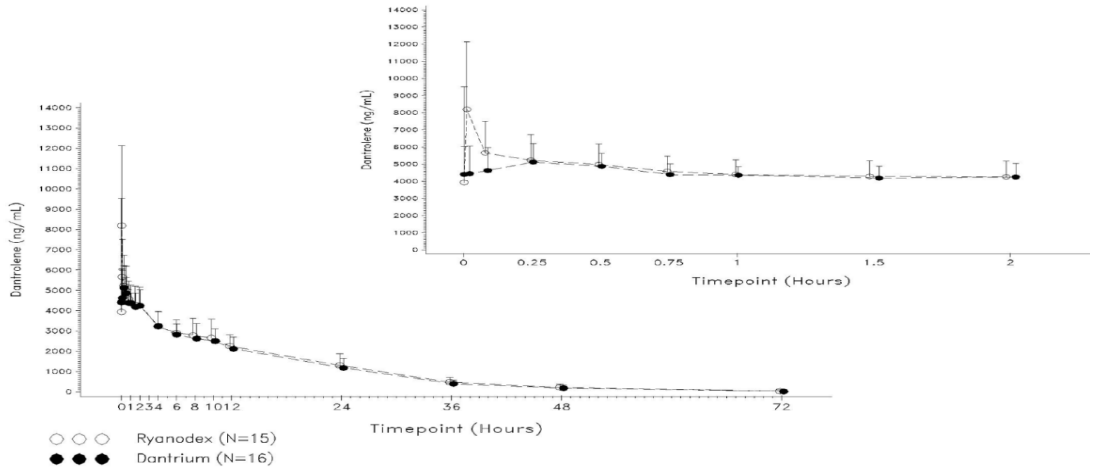
	1.0 mg/kg		1.75 mg/kg		2.0 mg/kg		2.25 mg/kg	
	A (N= 3)	B (N= 3)	A (N= 4)	B (N= 4)	A (N= 4)	B (N= 4)	A (N= 4)	B (N= 4)
$AUC_{0\text{-}INF}$_obs (hr*ug/mL)								
n	3	3	4	4	4	4	4	4
Mean	18.04	17.87	32.74	34.42	43.53	45.03	54.72	47.82
(SD)	(8.039)	(1.991)	(8.914)	(11.118)	(13.179)	(15.663)	(20.394)	(34.353)
$AUC_{0\text{-}last}$ (hr*ug/mL)								
n	3	3	4	4	4	4	4	4
Mean	16.70	16.75	30.89	32.74	41.89	44.16	53.47	45.77
(SD)	(7.782)	(1.802)	(9.065)	(11.358)	(12.444)	(15.408)	(20.524)	(33.307)
C_{max} (ng/mL)								
n	3	3	4	4	4	4	4	4
Mean	2713.33	2560.00	5445.00	3512.50	7125.00	3750.00	7452.50	3967.50
(SD)	(1416.557)	(669.029)	(3513.797)	(1745.802)	(1097.345)	(1524.620)	(1263.497)	(688.301)
$T_{1/2}$ (hr)								
n	3	3	4	4	4	4	4	4
Mean	9.48	11.40	9.50	10.71	9.86	8.68	8.52	8.87
(SD)	(1.926)	(1.797)	(1.719)	(1.978)	(2.529)	(2.723)	(2.042)	(4.581)
T_{max} (hr)								
n	3	3	4	4	4	4	4	4
Median	0.02	0.00	0.07	0.42	0.02	0.26	0.02	0.25
Min	0.0	0.0	0.0	0.0	0.0,	0.0	0.0,	0.0
Max	0.3	0.0	1.5	2.0	0.0	2.0	0.1	0.5

Data Source: Table 14.2.2

Part 2 PK: Comparison of Dantrolene PK following administration of Ryanodex and Dantrium.

In part 2 of the PK study conducted by CCD, 2.5 mg/kg dose or Ryanodex and Dantrium were administered to approximately 15 subjects in each arm. Whereas doses of 1.0 to 2.25 mg/kg in Part 1 were administered to male subjects only and the dose of 2.5 mg/kg was administered to both male (N=9) and female subjects (N=6).

Figure Mean (±SD) Dantrolene Plasma Concentrations vs. Time after Administration of 2.5 mg/kg Ryanodex or Dantrium



Source: Figure 14.2.2.1

Table: Summary of Dantrolene Plasma PK Parameters following IV administration of Ryanodex and Dantrium.

	2.5 mg/kg	
	Ryanodex (N= 15)	**Dantrium** (N= 16)
$AUC_{0\text{-}INF\_obs}$ (hr*ug/mL)		
n	15	16
Mean	77.72	71.61
(SD)	(23.154)	(19.066)
$AUC_{0\text{-}last}$ (hr*ug/mL)		
n	15	16
Mean	74.93	69.88
(SD)	(22.960)	(18.618)
C_{max} (ng/mL)		
n	15	16
Mean	8978.00	5715.63
(SD)	(4635.901)	(1269.672)
$T_{1/2}$ (hr)		
n	15	16
Mean	10.75	9.66
(SD)	(2.166)	(2.407)
T_{max} (hr)		
n	15	16
Median	0.02	0.25
Min	0.0	0.0
Max	1.0	1.5

Data Source: Table 14.2.2

Relative Bioavailability: For an intravenous product bioavailability is 100%. Since the two treatments, Ryanodex and Dantrium, were administered in a cross-over fashion in healthy volunteers, comparison of bioavailability was possible mathematically because of the difference in the duration of administration (bolus vs. infusion). The sponsor evaluated relative bioavailability of Dantrolene and 5-hydroxydantrolene, based on Cmax and $AUC_{0\text{-}inf}$ in all subjects that received both treatments of 2.5 mg/kg Ryanodex and Dantrium in the part 2 of the study. A summary statistical analysis is presented in the table below. For dantrolene, the 90% confidence intervals (CI) demonstrated that the two treatments were equivalent for AUC0-inf (using a 90% CI criteria of 80-125%). Significant differences between Ryanodex and Dantrium were evident for Cmax as the 90% CI range was 1.18-1.75. **This is likely a direct result of dosage form difference in dantrolene administration rate (mg/min).** Since Ryanodex doses were administered as a 60 second continuous IV push, and all Dantrium doses were administered as a infusion with a range of duration from 4 to 13 mins, it is reasonable that Ryanodex group had a higher Cmax value.

The relative bioavailability results demonstrate that $AUC_{0\text{-}inf}$ and C_{max} were 6% and 44% higher for Ryanodex as compared to Dantrium based on the GMR.

Table Relative Bioavailability Results for Dantrolene and 5-Hydroxydantrolene

	Ryanodex			Dantrium			Ryanodex/Dantrium	
Analyte/Parameter	N	GM	95% CI	N	GM	95% CI	GMR	90% CI
Dantrolene								
$AUC_{0\text{-}inf}$ (hr*ug/mL)	15	74.5	(63.0,88.1)	15	70.3	(60.5,81.7)	1.06	(0.99,1.14)
C_{max} (ng/mL)	15	7960	(6090,10400)	15	5530	(4940,6180)	1.44	(1.18,1.75)
5-hydroxydantrolene								
$AUC_{0\text{-}inf}$ (hr*ug/mL)	14[a]	20.2	(17.0,24.1)	14[a]	19.2	(16.2,22.7)	1.05	(0.99,1.13)
C_{max} (ng/mL)	15	593	(477,737)	15	602	(483,749)	0.99	(0.90,1.08)

GM=Geometric Mean; GMR= Geometric Mean Ratio
A linear mixed effects model is used with fixed effects terms for treatment and period. A log transformation is applied to the $AUC_{0\text{-}inf}$ and C_{max} data. Back-transformed summary statistics and inferential results are reported for pharmacokinetic parameters.
[a] $AUC_{0\text{-}inf}$ for 5-hydroxydantrolene was only estimable in N=14 subjects; this exposure parameter was not calculable for female subject 6002, based on available data for this analyte.

For the metabolite 5-hydroxydantrolene, AUC0-inf and Cmax estimates were comparable between Ryanodex and Dantrium as the 90% CI were within the 80-125% equivalence criteria.

OSI Inspection Results:

Because the study was the sole clinical study supporting the clinical experience with Ryanodex, Office of Scientific Investigations was consulted to conduct an inspection of the clinical site (CCD, Tacoma, WA) and bioanalytical site (b) (4) OSI review indicates that there were some plasma samples that showed possible hemolysis as concluded from red color in the plasma sample. The OSI review also notes that suspected hemolysis was not noted in all plasma samples and also they could not conclude a correlation between high dantrolene drug concentration and hemolysis. For example, some hemolysis was noted in several hours post-dose plasma samples. OSI reviewer (dated 6/19/2014 in Darrts) concluded as follows:

1. The clinical data from study EGL-Dantrolene-1201 are acceptable for review.

2. The bioanalytical data from study EGL-Dantrolene-1201 are acceptable for review if no significant correlation between hemolyzed samples and high dantrolene concentrations is confirmed by hemolyzed samples to be identified from the sponsor.

The sponsor was sent an information request to address hemolysis in the plasma samples collected for the Part 2 of the PK study where subjects received Ryanodex and Dantrium in a crossover fashion. A total of 1,156 plasma samples were submitted for visual sample color grading and evaluation of hemolytic index (HI). The sponsor's findings were summarized below.

Results of visual sample color grading:

A total of 1,112 (of the 1,156) samples were noted as "yellow" (i.e., normal plasma color), 43 samples were noted as "orange", and one sample was noted as "red".

Results of Hemolytic Index assessment:

Hemolytic index was determined for 1,125 (of the 1,156 samples in total) samples; 1,068 samples returned a HI value of 0, 51 samples returned a HI value of 1, 5 samples a HI value of 2, and only 1 sample returned a HI of 3. For the remaining 31 samples a HI value of 6 was returned in a repeat analysis and, these samples were visually inspected in accordance with SOP-CLP-2. Each of these 31 samples was determined to have no visual evidence of hemolysis. The independent sample color grading result recorded for these 31 samples showed 28 samples to be "yellow" in color and 3 samples to be "orange".

A total of 1,156 human plasma samples were analyzed for Dantrolene and 5-Hydroxydantrolene using a validated bioanalytical method.

The established bioanalytical method already examined the matrix effect with special consideration of hemolysis in plasma samples. As discussed in the bioanalytical method validation, the matrix effect was not found to be significant. In addition, the sponsor indicates that reproducibility of incurred samples was performed and the results met acceptance criteria (3.57% of the dantrolene ISR sample results and 13.8% of the 5-hydroxydantrolene ISR sample results differed from the original result by greater than

20%). The results from calibration curve standards and quality control samples demonstrated acceptable performance of the method for all reported concentrations.

A list of all samples suspected of hemolysis is presented in section 4.2.2 of the review. There was no pattern to the hemolysis noted in the plasma samples; there was no significant correlation between hemolyzed samples and high dantrolene concentrations. Small amount of hemolysis was observed irrespective of Dantrium or Ryanodex administration, in a random number of samples at different time points after drug administration (See section 4.2.2).

Based on the reasons described above, the PK results from study EGL-Dantrolene-1201 are acceptable for review.

Labeling:

The sponsor should edit the section 12.3 Pharmacokinetics to provide Ryanodex product specific pharmacokinetic information.

Conclusion:

Overall, the clinical pharmacology submission is acceptable.

2 QBR

2.1 General Attributes

Eagle Pharmaceuticals Inc., submitted NDA 205579 to market Dantrolene Sodium Suspension (250 mg/ 5mL vial) for the (b) (4) and treatment of Malignant Hyperthermia. Malignant Hyperthermia (MH) is an orphan disease and the sponsor obtained an orphan designation for the product (Designation number 03-1797). Previously approved Dantrium Infusion (IV) was used to treat MH with limitations on getting adequate therapeutic benefit.

Dantrium Intravenous (dantrolene sodium for injection) which contains 20 mg of dantrolene sodium per vial is the currently marketed product for the treatment of malignant hyperthermia (MH). The labeled dose of dantrolene sodium for an MH crisis ranges from 1 mg/kg to 10 mg/kg. Therefore, the dose for a 70 kg patient can reach 700 mg. This dose would require a total of 35 vials to be reconstituted immediately, each with 60 mL of WFI with a total volume to be rapidly administered exceeding 2 liters.

The sponsor contends that a nano-particle suspension offers a high concentration for delivery, coupled with the opportunity for rapid dissolution of the particles upon dilution in the blood due to their large surface area. A product presentation of 250 mg dantrolene sodium per vial to be reconstituted with 5 mL of water for injection was chosen to accommodate the dose range of 1 to 10 mg/kg. This presentation would require less than 3 vials and less than 15 mL to deliver a dose of 700 mg.

2.2 General Clinical Pharmacology

Although bioavailability of IV product is apparent and 100%, the sponsor conducted a clinical pharmacokinetic study to document safety, tolerability and PK of Ryanodex. The sponsor chose to compare the safety and tolerability of Ryanodex with Dantrium in a very limited number of subjects in a dose-escalation scheme. This was followed by a comparison of safety, tolerability and PK of Ryanodex with Dantrium at the proposed dose of 2.5 mg/kg.

- Part 1 was a dose escalation design where each treatment group received either Ryanodex or Dantrium at doses of 1.0, 1.75, 2.0, 2.25 or 2.5 mg/kg.
- Part 2 was conducted as a randomized, two-way crossover; subjects received 2.5 mg/kg of Ryanodex or Dantrium. Doses of 1.0 to 2.25 mg/kg were administered to male subjects (only) and the dose of 2.5 mg/kg was administered to both male and female subjects.

2.3 Intrinsic and Extrinsic Factors

Very few healthy subjects of different race, ethnicity were recruited in the study. The available data did not facilitate a review of PK data with regard to any influence of intrinsic or extrinsic factors. Most of the healthy subjects recruited in part 1 of the PK conducted by CCD were healthy male adults. However, about six healthy women were recruited in Part 2 of the PK study where healthy adult subjects did receive Dantirum and Ryanodex in a crossover fashion (See Demographics in the table below).

	A:B (N=8)	B:A (N=7)	Total (N=15)
Age (years)			
n	8	7	15
Mean	27.5	33.3	30.2
(SD)	(4.57)	(6.40)	(6.07)
Median	28.5	35.0	31.0
Min,	22	23	22
Max	33	42	42
Gender (%)			
Female	3 (38)	3 (43)	6 (40)
Male	5 (63)	4 (57)	9 (60)
Race (%)			
American Indian or Alaska Native	0	0	0
Asian	1 (13)	1 (14)	2 (13)
Black or African American	3 (38)	2 (29)	5 (33)
Native Hawaiian/Pacific Islander	0	0	0
Other	0	0	0
White	4 (50)	4 (57)	8 (53)
Ethnicity (%)			
Hispanic or Latino	2 (25)	1 (14)	3 (20)
Not Hispanic or Latino	6 (75)	6 (86)	12 (80)

Height (cm)	A:B (N=8)	B:A (N=7)	Total (N=15)
n	8	7	15
Mean	170.15	168.77	169.51
(SD)	(9.560)	(13.585)	(11.194)
Median	169.70	170.30	170.30
Min,	158.1,	150.1,	150.1,
Max	183.4	188.0	188.0
Weight (kg)			
n	8	7	15
Mean	70.73	67.24	69.10
(SD)	(10.963)	(15.914)	(13.110)
Median	70.65	60.00	68.40
Min,	57.8,	50.6,	50.6,
Max	86.8	93.2	93.2
BMI (kg/m^2)			
n	8	7	15
Mean	24.28	23.31	23.83
(SD)	(2.386)	(2.283)	(2.308)
Median	24.25	23.50	23.60
Min,	21.2,	20.2,	20.2,
Max	28.3	26.4	28.3

Some subjects could be from Part 1
A: Ryanodex, B: Dantrium
Data Source: Listing 16.2.4.1

The sponsor presented pharmacokinetic parameters as it relates exposure seemed to be higher in women compared to men at the first look in both Dantrium and Ryanodex treatments (See table below). This observation is despite the bodyweight based dosing (2.5 mg/kg) of both Dantrium and Ryanodex.

First, the number of females in the study was very limited (n=6).

Table Summary of Pharmacokinetic Parameters for Dantrolene by Gender for Ryanodex or Dantrium at 2.5 mg/kg

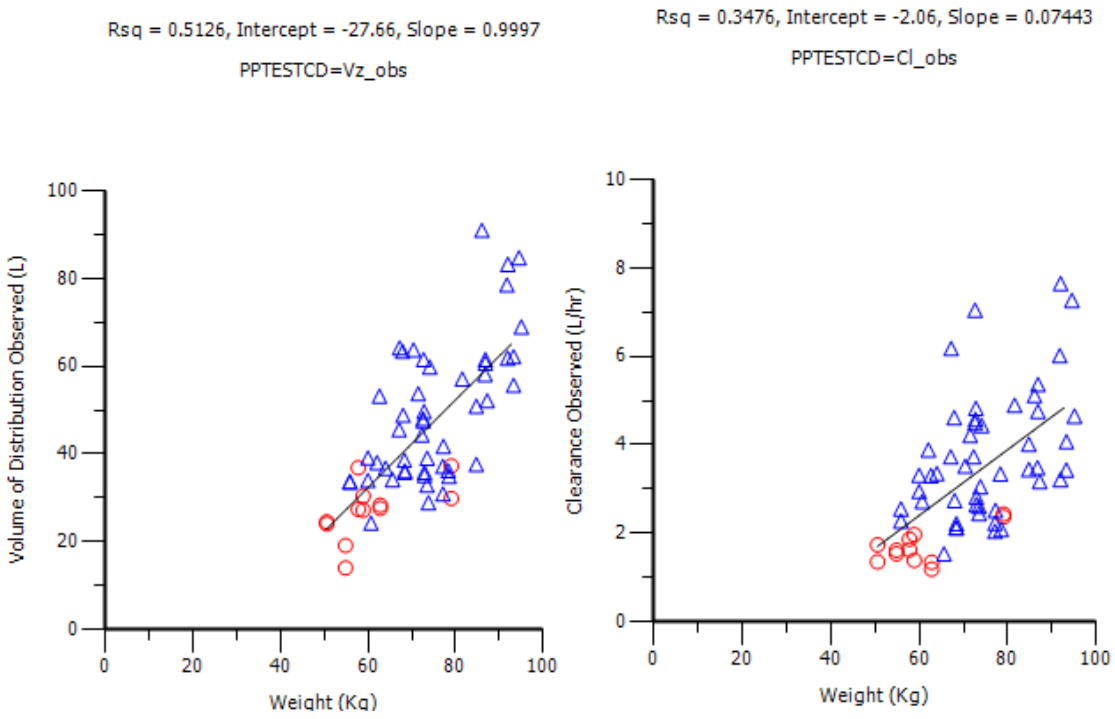
		Gender*									
		Female					Male				
Treatment		$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ug*hr/mL)	$AUC_{0\text{-inf}}$ (ug*hr/mL)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ug*hr/mL)	$AUC_{0\text{-inf}}$ (ug*hr/mL)
Ryanodex	N	6	6	6	6	6	9	9	9	9	9
	Mean	12.174	0.175	10976.667	96.629	99.316	9.8	0.024	7645.556	60.459	63.318
	SD	2.551	0.404	5217.857	15.630	16.359	1.267	0.022	3949.766	13.459	13.702
	Min	8.64	0	5470	82.528	86.218	8.19	0.02	3470	39.756	45.279
	Median	12.08	0.02	10280	91.215	93.297	9.36	0.02	6240	61.898	65.545
	Max	16.08	1	20200	125.587	130.111	11.54	0.08	16300	80.063	85.108
Dantrium	N	6	6	6	6	6	10	10	10	10	10
	Mean	10.54	0.461	6335	83.597	85.829	9.124	0.302	5344	61.644	63.072
	SD	3.18	0.554	1334.343	15.836	16.052	1.789	0.348	1135.089	15.414	15.714
	Min	6.02	0	4500	70.615	73.324	6.19	0	3750	39.168	40.855
	Median	10.13	0.38	6505	80.545	81.887	8.89	0.25	5095	57.112	57.925
	Max	14.55	1.5	7940	112.708	116.152	11.77	1	7130	89.877	92.914

Data Source: Listings 16.2.5.4 and 16.2.4.1
*** Data are arithmetically calculated from individual subject pharmacokinetic parameters, groupd by gender (male or female, per subject demographic information)**

Nevertheless, the PK parameters such as volume of distribution and clearance that are often affected by bodyweight were plotted with regard to the bodyweight of each subject. Volume of distribution and clearance data points for all doses were plotted over bodyweight of the subjects. As suspected, there was linear relationship between

bodyweight and volume of distribution or clearance of dantrolene. The limited range of bodyweights evaluated is a drawback.

Figure: Linear Plot of Volume of Distribution vs. Bodyweight (left) and Clearance (L/hr) Vs. Bodyweight (right). Data derived from males (triangles) from Part 1 and Part 2 and data derived from females (circles) from Part 2 only were plotted.



Partitioning to Red Blood Cells:

The sponsor evaluated binding of dantrolene to red blood cells (RBC). Incubations at 150 and 1503 ng/mL dantrolene sodium had 46% to 65% of dantrolene bound to RBCs, whereas incubations at 20,003 ng/mL dantrolene sodium had 27% to 36% dantrolene bound to RBCs (See section 4.2.3).

2.4 General Biopharmaceutics

Ryanodex is supplied as a sterile lyophilized powder containing 250 mg of dantrolene sodium [redacted], 125 mg of mannitol, 4 mg of povidone [redacted] and 25 mg of polysorbate 80 per vial, hydrochloric acid and sodium hydroxide.

2.5 Analytical

The bioanalytical method for the measurement of dantrolene and 5-hydroxydantrolene concentrations in human (K2EDTA) plasma

- Entails extraction of clinical plasma samples (100 µL aliquot) by supported-liquid extraction ([redacted]) or liquid-liquid extraction ([redacted]) methods prior to sample injection and
- Employs a liquid chromatography-mass spectroscopy (LCMS/MS) system (with an ionization source operated in negative ion mode) for analyte detection and quantification.

Multiple reaction monitoring transitions for analyte and internal standard detection are detailed in Table below.

Table Multiple Reaction Monitoring (MRM) Transitions for Analyte and Internal Standard Detection

	MRM Transitions Monitored (m/z)	
Laboratory Site	[redacted]	[redacted]
Analyte		
Dantrolene:	313 → 228	313 → 228
5-Hydroxydantrolene:	329 → 230	329 → 230
Internal Standard		
Dantrolene-$^{13}C_3$:	316 → 228	NA
Dantrolene-D_4:	NA	317 → 232
5-Hydroxydantrolene-D_4	333 → 234	333 → 234

NA = Not Applicable

The bioanalytical method was independently validated by two laboratory sites [redacted] & [redacted]) to support analyses of plasma samples from two clinical sites.

[redacted] Comprehensive Clinical Development, 3615 Pacific Avenue, Tacoma, WA 98419, respectively) for analyte concentration over a linear range, as detailed in Table below.

Table Analyte Calibration Range and Standard Curve Calculation Parameters

Laboratory Site	Analyte and Calibration Range (ng/mL)				Regression Type (for quantitation of analytes)	Coefficient of Determination (r^2)	
	Dantrolene		5-Hydroxydantrolene			Dantrolene	5-Hydroxydantrolene
	LLOQ	ULOQ	LLOQ	ULOQ			
(b) (4)	50.0	50,000	10.0	10,000	Linear $1/x^2$ [y= ax + b]	0.9980	0.9981
(b) (4)	50.0	25,000	10.0	5,000	Quadratic 1/x [$y=(a)x^2 = bx + c$]	0.9992	0.9986

LLOQ = Lower Limit of Quantitation; ULOQ = Upper Limit of Quantitation
Source: (b) (4) 3006230, (b) (4) 1773-021

Method selectivity was assessed by evaluation of cross-analyte and -internal standard interference, as well as due to matrix (across multiple lots; also including hemolyzed and lipemic conditions) and presence of common over-the-counter medications, and no significant interferences were observed (Assay Selectivity in 3006230 & Validation Summary in 1773-021). There was no significant suppression or enhancement of analyte ion signal as a result of matrix interference (mean internal standard-normalized matrix factor relative standard deviation range of 0.79% to 4.03% for dantrolene and 1.56% to 5.16% for 5-hydroxydantrolene). Detection specificity was assessed at the liquid chromatography retention times of dantrolene, 5-hydroxydantrolene and internal standards and identified the absence of any interference in blank human plasma. Extraction recovery of all analytes and internal standards from blank matrix was 84% to 103%. Precision and accuracy at the lower limit of quantitation (LLOQ) and at low, middle and high quality control (QC) dantrolene and 5-hydroxydantrolene concentration levels were evaluated and met acceptance criteria that intra-run and inter-run precision and accuracy must be within 20.0% (of LLOQ)/15.0% (of QC). Assessment of sample dilution integrity (at 10x, 20x, & 100x dilution of QC samples) was conducted for both analytes and results met acceptance criteria of (intra-run) precision and accuracy to be within 15.0%. Analyte carryover and stock solution stability, sample analysis batch size determination, and sample reinjection reproducibility were also assessed and met guidance acceptance criteria.

Benchtop, freeze/thaw, and extract stability of dantrolene and 5-hydroxydantrolene in human plasma were evaluated at low and high quality control concentration levels for both analytes and found to be compatible with the conduct of blood sample collection, processing and storage in the clinical study. The long term storage (at -70˚C) stability of dantrolene and 5-hydroxydantrolene in human plasma is at least 143 days.

Human whole blood (K2EDTA) stability was performed for dantrolene and 5-hydroxydantrolene at quality control low, middle, and high concentration levels following up to 2 hours at ambient temperature or on wet ice. Results met acceptance criteria at the quality control high concentration level (for at least 1.5 hours) but failed at lower concentration levels. The available data suggest that analyte recovery from whole blood (and not stability in this matrix) is the reason for these results and do not impact the integrity of method validation or clinical study results and interpretation. The affinity of dantrolene to bind to red blood cells likely accounts for the lack of recovery of dantrolene (irrespective of storage temperature) at quality control low and middle concentration

levels, given the extensive partitioning of dantrolene to red blood cells (and resultant loss of analyte by centrifugation of cells during processing of whole blood to plasma, the medium used for concentration measurements). Based on assays of whole blood and plasma, greater amounts of dantrolene are associated with red blood cells than with plasma. The affinity for 5-hydroxydantrolene to bind red blood cells is unknown, but is estimated to be analogous to dantrolene given the structural similarity of these analytes. Thus, whole blood stability of dantrolene and 5-hydroxydantrolene was found to be compatible with the conduct of blood sample collection, processing and storage in the clinical study.

A partial cross validation was performed using quality control samples (dantrolene & 5-hydroxydantrolene concentrations: QC Low [150 & 30.0 ng/mL], QC Medium [2,500 & 500 ng/mL], QC High [40,000 & 8,000 ng/mL], QC Very High [250,000 & 50,000 ng/mL]) prepared and qualified during the (b) (4) bioanalytical method validation (3006230). These QC samples (supplied by (b) (4)) were analyzed as a part of the (b) (4) bioanalytical method validation (Table 5 in 1773-021) and were found to be within the acceptance criteria (i.e. mean intra-run accuracy within ±20.0% of the nominal concentration and precision ≤20.0%). These results suggest that the (b) (4) method is comparable to the (b) (4) method for the accurate quantification of dantrolene and 5-hydroxydantrolene in clinical plasma samples.

The method validation study results indicated that the bioanalytical method is sensitive, selective, accurate, and reproducible for the detection and quantitation of dantrolene and 5-hydroxydantrolene in human plasma and demonstrate that these analytes are stable during storage, processing, and analysis as conducted in the clinical study.

OSI Inspection Results:

Because the study was the sole clinical study supporting the clinical experience with Ryanodex, Office of Scientific Investigations was consulted to conduct an inspection of the clinical site (CCD, Tacoma, WA) and bioanalytical site ((b) (4)). OSI review indicates that there were some plasma samples that showed possible hemolysis as concluded from red color in the plasma sample. The review also notes that suspected hemolysis was not noted in all plasma samples and also they could not conclude a correlation between high dantrolene drug concentration and hemolysis. For example, some hemolysis was noted in several hours post-dose plasma samples. OSI reviewer concluded as follows:

1. The clinical data from study EGL-Dantrolene-1201 are acceptable for review.

2. The bioanalytical data from study EGL-Dantrolene-1201 are acceptable for review if no significant correlation between hemolyzed samples and high dantrolene concentrations is confirmed by hemolyzed samples to be identified from the sponsor.

The sponsor was sent an information request to address hemolysis in the plasma samples collected for the Part 2 of the PK study where subjects received Ryanodex and Dantrium in a crossover fashion. A total of 1,156 plasma samples were submitted for visual sample color grading and evaluation of hemolytic index (HI).

Results of visual sample color grading:

A total of 1,112 (of the 1,156) samples were noted as “yellow” (i.e., normal plasma color), 43 samples were noted as “orange”, and one sample was noted as “red”.

Results of Hemolytic Index assessment:

Hemolytic index was determined for 1,125 (of the 1,156 samples in total) samples; 1,068 samples returned a HI value of 0, 51 samples returned a HI value of 1, 5 samples a HI value of 2, and only 1 sample returned a HI of 3. For the remaining 31 samples a HI value of 6 was returned in a repeat analysis and, these samples were visually inspected in accordance with SOP-CLP-2. Each of these 31 samples was determined to have no visual evidence of hemolysis. The independent sample color grading result recorded for these 31 samples showed 28 samples to be “yellow” in color and 3 samples to be “orange”.

A total of 1,156 human plasma samples were analyzed for Dantrolene and 5-Hydroxydantrolene using a validated bioanalytical method.

The established bioanalytical method already examined the matrix effect with special consideration of hemolysis in plasma samples. As discussed in the bioanalytical method validation, the matrix effect was not found to be significant. In addition, the sponsor indicates that reproducibility of incurred samples was performed and the results met acceptance criteria (3.57% of the dantrolene ISR sample results and 13.8% of the 5-hydroxydantrolene ISR sample results differed from the original result by greater than 20%). The results from calibration curve standards and quality control samples demonstrated acceptable performance of the method for all reported concentrations.

A list of all samples suspected of hemolysis are presented in section 4.2.2 of the review. There was no pattern to the hemolysis noted in the plasma samples. Small amount of hemolysis was observed irrespective of Dantrium or Ryanodex administration, in a random number of samples at different time points after drug administration (See section 4.2.2).

4.2 Individual Study Reviews

4.2.1 Bioanalytical method validation:

VALIDATION SUMMARY

Objective:	This study was conducted to validate an LC-MS/MS method for the analysis of Dantrolene and 5-Hydroxydantrolene in human plasma with K_2EDTA.
Study Initiation Date:	January 17, 2013
Validation Period:	January 25, 2013 to August 29, 2013
Analytes:	Dantrolene (Appendix A) Lot Number: F0G156 Correction Factor: (b) (4); corrected for sodium salt content (b) (4) Storage: Room temperature, protected from light 5-Hydroxydantrolene (Appendix A) Lot Number: 1-MHS-135-2 Correction Factor: (b) (4); corrected for purity (b) (4) Storage: In a freezer set to -20°C 5-Hydroxydantrolene (Appendix A) Lot Number: 1111-050A7 Correction Factor: (b) (4); corrected for purity (b) (4) Storage: In a refrigerator set to 4°C
Internal Standards:	Dantrolene-d4 (Appendix A) Lot Number: 1071-082A2 (b) (4) Storage: In a refrigerator set to 4°C, protected from light 5-Hydroxydantrolene-d4 (Appendix A) Lot Number: 1118-012A2 (b) (4) Storage: In a refrigerator set to 4°C
Data Collection and Analysis Software:	Analyst Version 1.4.2 (b) (4) Watson LIMS Version 7.4 (b) (4) ExyLIMS Version 3.0 (b) (4)
Method Description:	Each 100 µL aliquot of standard or sample was mixed with 50.0 µL of working IS solution (5000 ng/mL dantrolene-d4/1000 ng/mL 5-hydroxydantrolene-d4) and 500 µL of acetonitrile. The sample was vortexed and centrifuged, and 200 µL of the resulting supernatant was transferred to a clean black 96-well autosampler plate, combined with 200 µL of water and mixed. A 20 µL aliquot was injected onto an LC-MS/MS system for analysis.

LC-MS/MS Conditions:	The liquid chromatography system used an ACE 5 C_{18} column, 2.1 x 100 mm (5 µm particle size) with an gradient flow of 4.5 mM ammonium acetate in water/formic acid (100:0.1, v/v) and 4.5 mM ammonium acetate in water/acetonitrile/formic acid (45:55:0.045, v/v/v) at a flow rate of 0.500 mL/minute. The analyte and internal standard were detected using an (b) (4) (b) (4) LC-MS/MS system equipped with an ESI (TurboIonSpray®) ionization source operated in the negative ion mode. The following MRM transitions of the respective $[M-H]^-$ ions were used to monitor dantrolene, 5-hydroxydantrolene, dantrolene-d4, and 5-hydroxydantrolene-d4 and may have been slightly modified to optimize system response. Actual transitions and retention times were documented in the data. Transition Monitored — Retention Time Analyte Dantrolene: m/z = 313 → 228 — 2.0 – 3.1 min 5-Hydroxydantrolene: m/z = 329 → 230 — 1.9 – 3.0 min Internal Standard Dantrolene-d4: m/z = 317 → 232 — 2.0 – 3.1 min 5-Hydroxydantrolene-d4 m/z = 333 → 234 — 2.2 – 2.6 min
Regression Type:	Quadratic 1/x concentration weighting $y = (a)x^2 + bx + c$
Calibration Range:	Dantrolene: 50.0 (LLOQ) to 25,000 (ULOQ) ng/mL 5-Hydroxydantrolene: 10.0 (LLOQ) to 5,000 (ULOQ) ng/mL
Calibration Standard Storage:	Prepared fresh daily, with the exception of calibration standards extracted for validation run 18 on April 4, 2013, which were stored in a freezer set to -70°C for 29 days until use.
QC Preparation Dates and Storage:	January 25, 2013: stored in a freezer set to -20°C or -70°C February 1, 2013: stored in a freezer set to -20°C or -70°C March 11, 2013: stored in a freezer set to -70°C March 13, 2013: stored in a freezer set to -70°C April 11, 2013: prepared fresh July 17, 2013: prepared fresh July 24, 2013: prepared fresh August 1, 2013: prepared fresh
Biological Matrix and Supplier:	Human plasma with K_2EDTA and whole blood with K_2EDTA from (b) (4) (b) (4) Hyperlipidemic plasma was provided by (b) (4) Hemolytic plasma was prepared by (b) (4) Whole blood procured from (b) (4) was used within the assigned expiration date. Whole blood procured from (b) (4) was used within one day of collection.
Validation Design:	Per Protocol and Amendments (Appendix B)

Acceptance Criteria	
Calibration Standards:	1. Accuracy within ±15.0% (±20.0% at LLOQ) of the nominal concentration. 2. A minimum of six standard levels and minimum of 75% of all calibration standards must be used in the regression. 3. Coefficient of determination (r^2) ≥0.985.
Quality Control Samples:	1. At least 66.7% of the QC samples in each run and at least 50% at each concentration level must have accuracy within ±15.0% (±20.0% at the LLOQ) of the nominal concentration. 2. Mean intra- and inter-run accuracy within ±15.0% (±20.0% at the LLOQ) of the nominal concentration and precision ≤15.0% (≤20.0% at the LLOQ).
Assessments	
Regression Evaluation:	Least aggressive model and weighting with r^2 value closest to 1.0000 was selected from linear and quadratic models evaluated with 1/x and $1/x^2$ weightings.
Cross Validation:	Mean intra-run accuracy within ±20.0% of the nominal concentration, and precision ≤20.0%.
Dilution:	At least 66.7% of the dilution QC samples must have accuracy within ±15.0% of the nominal concentration, mean accuracy within ±15.0% of the nominal concentration, and precision ≤15.0%.
Carryover:	1. Analyte response ≤20.0% of the mean analyte response in the acceptable LLOQ standards. 2. IS response ≤5.0% of the mean IS response in the acceptable LLOQ standards.
Selectivity:	1. Analyte response ≤20.0% of the mean analyte response in the acceptable LLOQ standards. 2. IS response ≤5.0% of the mean IS response in the acceptable LLOQ standards. 3. OTC QCs must have mean accuracy within ±15.0% of the nominal concentration and precision ≤15.0%.
Matrix Effect:	Precision of the IS normalized MF for the six lots must be ≤15.0%.
Recovery:	Precision for the analyte and IS must be ≤15.0% at each level, and recovery must be consistent within and across QC levels
Matrix Stability:	Plasma: Mean accuracy within ±15.0% of the nominal concentration and precision ≤15.0%. Whole Blood: Mean difference within ±15.0% of the nominal concentration and precision ≤15.0%. $$\%D = \frac{\text{mean of aged} - \text{mean of fresh}}{\text{mean of fresh}} x100$$

Stock Solution Stability:	Precision must be ≤5.0% RSD and mean difference within ±5.0% for analyte, precision must be ≤15.0% RSD and mean difference within ±15.0% for IS. $$\%D = \frac{\text{mean of aged} - \text{mean of fresh}}{\text{mean of fresh}} x100$$
Results and Discussion	
Validation Run Summary:	Table 1
Calibration Standards:	Table 2 Regression Type / Dantrolene r^2 / 5-Hydroxydantrolene r^2 Linear 1/x — 0.9987 — 0.9981 Linear $1/x^2$ — 0.9971 — 0.9958 **Quadratic 1/x — 0.9992 — 0.9986** Quadratic $1/x^2$ — 0.9976 — 0.9971 Six lots of matrix were evaluated including one lot with 2 to 3% hemolysis and one hyperlipemic lot. The simplest regression model with the coefficient of determination closest to 1.000 was selected. The initial regression and Day 1 of validation were rejected due to suspected contamination. Day 1 of validation was repeated using the same QC pool and failed to meet the acceptance criteria for dantrolene at the QC Mid level and for 5-hydroxydantrolene at the QC LLOQ and QC Mid. The QC LLOQ and QC Mid were re-prepared and the validation restarted without further incident (with noted exception below). Following repeated 5-hydroxydantrolene calibration standard and QC failures, an investigation was initiated and determined that the instrument, the column, and ion suppression could be eliminated as possible causes of the 5-hydroxydantrolene measurement failures. Based on the investigation results, the mitigating measure undertaken was to add a stable label IS for 5-hydroxydantrolene. 5-Hydroxydantrolene-d4 was successfully cross-validated into the current method, thus providing a more accurate detection and quantitation of this analyte. A representative calibration curve is presented in Figure 1.
QC Samples:	Tables 3 and 4
Dilution:	Table 3
Cross Validation:	Table 5 QC samples prepared and qualified using a method (Document Control Number 3006230) validated by (b) (4) were analyzed under the current method and were found to be within the acceptance criteria. Samples (dantrolene/5-hydroxydantrolene concentrations) included: QC Low (150 ng/mL/30.0 ng/mL), QC Medium (2,500/500 ng/mL), QC High (40,000/8,000 ng/mL), and QC Very High (250,000/50,000 ng/mL). These results suggest that the methods used at both (b) (4) are deemed comparable for the accurate quantification of dantrolene and 5- hydroxydantrolene.

Table 3 Quality Control Analysis Results - Dantrolene

Run Date	Run Number	QC LLOQ 50.0 ng/mL	QC Low 147 ng/mL	QC Low 150 ng/mL	QC Mid 1430 ng/mL	QC Mid 1500 ng/mL	QC High 19600 ng/mL	QC High 20000 ng/mL	QC Dilution 10x 20000 ng/mL	QC Dil 20x 47600 ng/mL	QC Dil 20x 50000 ng/mL	QC Dilution 100x 50000 ng/mL	QC Dilution 10x 50000 ng/mL
30-Jan-2013	8	47.8		154		1440		19500					
		45.3		161		1500		19900					
		48.8		157		1480		20000					
		48.9		163		1550		19700					
		53.0		160		1420		20200					
		45.0		151		1390		19500					
Intrarun Mean		48.1		158		1460		19800					
Intrarun SD		2.92		4.55		58.2		283					
Intrarun %RSD		6.1		2.9		4.0		1.4					
Intrarun %RE		-3.8		5.3		-2.7		-1.0					
n		6		6		6		6					
31-Jan-2013	7	52.9		157		1550		20700					
		53.4		157		1520		20900					
		56.2		155		1530		21200					
		51.4		156		1540		20200					
		55.9		158		1530		20200					
		49.7		158		1480		20100					
Intrarun Mean		53.3		157		1530		20600					
Intrarun SD		2.53		1.17		24.3		451					
Intrarun %RSD		4.7		0.7		1.6		2.2					
Intrarun %RE		6.6		4.7		2.0		3.0					
n		6		6		6		6					

Table 3 Quality Control Analysis Results - 5-Hydroxydantrolene

Run Date	Run Number	QC LLOQ 10.0 ng/mL	QC Low 30.0 ng/mL	QC Mid 300 ng/mL	QC High 4000 ng/mL	QC Dilution 10x 4000 ng/mL	QC Dil 20x 10000 ng/mL	QC Dilution 100x 10000 ng/mL
25-Jul-2013	25		27.6	288	4040			
			30.5	296	3980			
			28.8	301	3870			
			25.7	290	4020			
			29.5	290	4010			
			26.8	296	4050			
Intrarun Mean			28.2	294	4000			
Intrarun SD			1.78	4.97	66.0			
Intrarun %RSD			6.3	1.7	1.7			
Intrarun %RE			-6.0	-2.0	0.0			
n			6	6	6			
01-Aug-2013	28		30.0	294	4030			
			29.6	289	4010			
			31.0	286	4050			
			30.0	292	4080			
			28.5	283	4050			
			30.7	292	3820			
Intrarun Mean			30.0	289	4010			
Intrarun SD			0.882	4.18	94.4			
Intrarun %RSD			2.9	1.4	2.4			
Intrarun %RE			0.0	-3.7	0.3			
n			6	6	6			
~ > 15%RE								
~~ > 20%RE								

Table 4 Inter-day Quality Control Analysis Results - Dantrolene

Run Date	Run Number	QC LLOQ 50.0 ng/mL	QC Low 150 ng/mL	QC Mid 1500 ng/mL	QC High 20000 ng/mL
12-Apr-2013	20	~38.8	137	1450	20400
		~39.4	134	1480	20900
		~39.9	142	1410	20200
		44.8	137	1490	21000
		40.6	148	1530	20100
		42.1	151	1430	19200
Mean Concentration Found (ng/mL)		46.2	148	1460	19800
Inter-run SD		4.93	7.45	60.6	693
Inter-run %RSD		10.7	5.0	4.2	3.5
Inter-run %RE		-7.6	-1.3	-2.7	-1.0
Inter-run %Total Error		18.3	6.4	6.8	4.5
n		42	42	42	42
~ > 20%RE					

Table 5 Cross Validation Quality Control Analysis Results - Dantrolene

Run Date	Run Number	QCL 1875-O-1 150 ng/mL	QCM 1875-P-1 2500 ng/mL	QCH 1875-Q-1 40000 ng/mL	QC VH 1875-J-1 250000 ng/mL
19-Mar-2013	17	145	2530	39000	242000
		147	2620	36900	260000
		147	2450	37900	281000
		151	2510	38900	255000
		143	2470	38500	276000
		144	2530	38800	269000
Intrarun Mean		146	2520	38300	264000
Intrarun SD		2.86	59.5	807	14400
Intrarun %RSD		2.0	2.4	2.1	5.5
Intrarun %RE		-2.7	0.8	-4.3	5.6
n		6	6	6	6
12-Apr-2013	21	154	2610	44000	251000
		144	2640	46200	250000
		149	2650	41500	282000
		146	2750	45300	254000
		155	2780	41200	277000
		154	2670	40500	267000
Intrarun Mean		150	2680	43100	264000
Intrarun SD		4.68	66.8	2370	13900
Intrarun %RSD		3.1	2.5	5.5	5.3
Intrarun %RE		0.0	7.2	7.8	5.6
n		6	6	6	6

Table 5 Cross Validation Quality Control Analysis Results - 5-Hydroxydantrolene

Run Date	Run Number	QCL 1875-O-1 30.0 ng/mL	QCM 1875-P-1 500 ng/mL	QCH 1875-Q-1 8000 ng/mL	QC VH 1875-J-1 50000 ng/mL
12-Apr-2013	21	34.8	587	8780	53800
		31.0	582	9580	55300
		30.6	564	9100	~~61600
		32.2	570	9570	55400
		30.4	581	9170	58000
		33.5	538	8530	52600
Intrarun Mean		32.1	570	9120	56100
Intrarun SD		1.77	18.0	420	3240
Intrarun %RSD		5.5	3.2	4.6	5.8
Intrarun %RE		7.0	14.0	14.0	12.2
n		6	6	6	6
~~ > 20%RE					

Carryover:	Table 6 Analyte response in the carryover blanks was observed in runs 8 to 10 and run 26 for dantrolene and runs 9 and 10 for 5-hydroxydantrolene. Carryover was confirmed in runs 9 and 10 and inspection of the autosampler system showed that the needle wash was not being properly drawn. Needle wash draw was corrected prior to the restart of run 11, which had no evidence of carryover. Run 26 was completed as multiple reinjections from run 25 for reinjection reproducibility and the response observed in the second carryover blanks in run 26 may be related to contamination following multiple reinjections of the same double blank sample throughout runs 25 and 26. Dantrolene-d4 internal standard response was observed in runs 12 and 15 due to the inadvertent inclusion of the blank with IS (dantrolene-d4) sample after the ULOQ. Due to the sporadic nature and confirmed reason for the analytes or IS (dantrolene-d4 and 5-hydroxydantrolene-d4) response it was concluded that carryover is not a method limitation.
Selectivity:	Table 7: Six lots of human plasma with K_2EDTA (control matrix) were evaluated. There was no significant interference observed from endogenous components or other sources at the mass transitions and retention times of the analyte or IS with the exception of the initial evaluation (run 7) of the selectivity lot containing over the counter (OTC) compounds. This observed response (>20.0% of the mean LLOQ analyte peak area response) was not in alignment with the OTC spiked QC Low and High concentration levels which met the acceptance criteria. The OTC selectivity assessment was repeated in duplicate (run 9) and responses were found to be within acceptance criteria. Thus, the aberrant response measured in run 7 was suspected to be the result of contamination during sample preparation.
Matrix Effect:	Table 8: There was no significant suppression or enhancement of analyte ion signal as a result of matrix interference (mean IS normalized MF range of 0.965 to 1.11).
Recovery:	Table 9: Recovery of dantrolene, 5-hydroxydantrolene and dantrolene-d4 were consistent (recovery ranged from 92.5% to 103%). Recovery of 5-hydroxydantrolene-d4 was not required to be assessed by protocol. The recovery for the d4 stable label of the metabolite is assumed to be similar to 5-hydroxydantrolene due to a molecular weight difference of only 4.
Reinjection Reproducibility:	Table 10: Both analytes were stable up to 116 injections at ambient temperature. Reinjection reproducibility was initially assessed in run 24 and failed to meet the acceptance criteria due to insufficient sample for reinjection. The assessment was repeated in run 26 and met all acceptance criteria.
Freeze/Thaw Cycle Stability:	Table 11: Analytes were considered stable after 3 freeze/thaw cycles at -20°C and -70°C.
Bench Top Stability:	Table 12: Dantrolene was stable for 25 hours at ambient temperature and 5-hydroxydantrolene was stable for 20 hours at ambient temperature.

Table 7 Selectivity Analysis Results - Dantrolene

Run Date	Run Number	OTC QC Low 150 ng/mL	OTC QC High 20000 ng/mL
31-Jan-2013	7	137	19800
		148	19900
		147	19700
		156	19600
		149	19700
		143	19800
Intrarun Mean		147	19800
Intrarun SD		6.35	105
Intrarun %RSD		4.3	0.5
Intrarun %RE		-2.0	-1.0
n		6	6

Table 7 Selectivity Analysis Results - 5-Hydroxydantrolene

Run Date	Run Number	OTC QC Low 30.0 ng/mL	OTC QC High 4000 ng/mL
31-Jan-2013	7	28.1	4350
		31.0	4410
		31.4	4280
		30.2	4310
		30.4	4300
		27.8	4300
Intrarun Mean		29.8	4330
Intrarun SD		1.51	47.6
Intrarun %RSD		5.1	1.1
Intrarun %RE		-0.7	8.3
n		6	6

Table 8 **Matrix Effect Results - Dantrolene**

Run Number	Matrix Lot	Peak Area: Blank Spiked Matrix	Peak Area: Neat Solution	Peak Area: Mean Neat Solution	Matrix Factor	Mean Matrix Factor	%RSD
9	1	8814.65	8184.84	8657.37	1.02	1.05	3.03
	2	8957.00	8970.23		1.03		
	3	8792.97	8651.01		1.02		
	4	9057.05	8955.92		1.05		
	5	9205.75	8379.87		1.06		
	6	9521.54	8802.35		1.10		

Matrix Effect Results – Internal Standard

Matrix Lot	Peak Area: Blank Spiked Matrix	Peak Area: Neat Solution	Peak Area: Mean Neat Solution	Matrix Factor	Mean Matrix Factor	%RSD
1	113941	116072	112461	1.01	1.05	3.26
2	115228	111371		1.02		
3	117401	108010		1.04		
4	115927	112181		1.03		
5	121136	114435		1.08		
6	123936	112697		1.10		

Matrix Effect Results – IS Normalized

Matrix Lot	Analyte MF	IS MF	IS Normalized MF	Mean IS Normalized MF	%RSD
1	1.02	1.01	1.00	1.00	1.56
2	1.03	1.02	1.01		
3	1.02	1.04	0.973		
4	1.05	1.03	1.01		
5	1.06	1.08	0.99		
6	1.10	1.10	1.00		

Table 8 **Matrix Effect Results – 5-Hydroxydantrolene**

Run Number	Matrix Lot	Peak Area: Blank Spiked Matrix	Peak Area: Neat Solution	Peak Area: Mean Neat Solution	Matrix Factor	Mean Matrix Factor	%RSD
9	1	5019.47	4535.65	4635.64	1.08	1.11	6.38
	2	4957.75	4748.59		1.07		
	3	4672.15	4471.92		1.01		
	4	5114.95	4776.65		1.10		
	5	5565.99	4450.11		1.20		
	6	5434.93	4830.92		1.17		

Matrix Effect Results – Internal Standard

Matrix Lot	Peak Area: Blank Spiked Matrix	Peak Area: Neat Solution	Peak Area: Mean Neat Solution	Matrix Factor	Mean Matrix Factor	%RSD
1	113941	116072	112461	1.01	1.05	3.26
2	115228	111371		1.02		
3	117401	108010		1.04		
4	115927	112181		1.03		
5	121136	114435		1.08		
6	123936	112697		1.10		

Matrix Effect Results – IS Normalized

Matrix Lot	Analyte MF	IS MF	IS Normalized MF	Mean IS Normalized MF	%RSD
1	1.08	1.01	1.07	1.05	4.69
2	1.07	1.02	1.04		
3	1.01	1.04	0.965		
4	1.10	1.03	1.07		
5	1.20	1.08	1.11		
6	1.17	1.10	1.06		

Table 10 Reinjection Reproducibility Results - Dantrolene Ambient

Run Date	Run Number	QC Low R1 150 ng/mL	QC Low R3 150 ng/mL	QC High R1 20000 ng/mL	QC High R3 20000 ng/mL
25-Jul-2013	26	154	144	21000	20300
		137	136	19700	19500
		144	144	19600	19700
		149	145	19700	19500
		140	140	20300	20400
		139	136	20000	19800
Mean		144	141	20100	19900
S.D.		6.55	4.12	532	393
%RSD		4.5	2.9	2.6	2.0
%Nominal		96.0	94.0	100.5	99.5
%RE		-4.0	-6.0	0.5	-0.5
%Total Error		8.5	8.9	3.1	2.5
n		6	6	6	6

Table 10 Reinjection Reproducibility Results - 5-Hydroxydantrolene Ambient

Run Date	Run Number	QC Low R1 30.0 ng/mL	QC Low R3 30.0 ng/mL	QC High R1 4000 ng/mL	QC High R3 4000 ng/mL
25-Jul-2013	26	29.1	29.9	4090	4140
		31.1	27.9	3860	3950
		30.0	29.4	3980	3970
		31.4	30.2	3980	3960
		29.5	28.4	4090	4030
		29.0	28.4	4040	4060
Mean		30.0	29.0	4010	4020
S.D.		1.02	0.931	87.1	73.6
%RSD		3.4	3.2	2.2	1.8
%Nominal		100.0	96.7	100.3	100.5
%RE		0.0	-3.3	0.3	0.5
%Total Error		3.4	6.5	2.4	2.3
n		6	6	6	6

Table 11 Freeze Thaw Cycle Stability Results - Dantrolene -20°C

Run Date	Run Number	3 cycle QC Low 150 ng/mL	3 cycle QC High 20000 ng/mL
30-Jan-2013	8	148	20200
		149	20300
		143	19900
		150	20300
		146	20200
		158	20200
Mean		149	20200
S.D.		5.06	147
%RSD		3.4	0.7
%Nominal		99.3	101.0
%RE		-0.7	1.0
%Total Error		4.1	1.7
n		6	6

Table 12 Bench Top Stability Results - Dantrolene Ambient

Run Date	Run Number	25 hour QC Low 150 ng/mL	25 hour QC High 20000 ng/mL
30-Jan-2013	8	145	20000
		147	20200
		147	19500
		148	20300
		156	19800
		151	19600
Mean		149	19900
S.D.		3.95	322
%RSD		2.7	1.6
%Nominal		99.3	99.5
%RE		-0.7	-0.5
%Total Error		3.3	2.1
n		6	6

Long Term Storage Stability:	Table 13: Dantrolene at -20°C: Assessed at 53, 77, 173, and 188 days Dantrolene at -70°C: Assessed at 53, 77, 143, 147, and 173 days 5-Hydroxydantrolene at -20°C: Assessed at 77, 173, and 188 days 5-Hydroxydantrolene at -70°C: Assessed at 77, 143, 147, and 173 and days; DNMT at 147 or 173 days The initial long term stability assessment at 173 days failed to meet the acceptance criteria for 5-hydroxydantrolen stored at -70°C for %RSD due to one sample with approximately 50% higher response than all other replicates. Long term stability was reassessed at -70°C using a different batch of QC samples that had been stored for up to 147 days and failed to meet the acceptance criteria at both QC levels due to a high bias in the stability samples. The QCs that were used for the second of these two stability assessments (147 day) had been previously shown to be prepared with a high bias and should have been discarded and unavailable for use in the long term stability assessment. The remaining QCs from these pools have subsequently been discarded. Therefore, stability was reassessed in run 28 (188 days at -20°C and 143 days at -70°C) with a QC pools that had been shown to be within 4% of nominal. All criteria were met for dantrolene and 5-hydroxydantrolene in run 28. Both analytes were stable up to 188 days at -20°C. Dantrolene was stable up to 173 days at -70°C and 5-hydroxydantrolene was stable up to 143 days at -70°C.
Post-processed Stability:	Table 14: Both analytes were stable for 90 hours at ambient temperature.
Autosampler Stability:	Table 15: Both analytes were stable for 79 hours at ambient temperature.

Table 13 Long Term Storage Stability Results - Dantrolene -70°C

Run Date	Run Number	143 day QC Low 150 ng/mL	143 day QC High 20000 ng/mL
01-Aug-2013	28	150	19300
		149	19600
		148	20100
		148	19600
		158	19900
		138	20200
Mean		149	19800
S.D.		6.38	343
%RSD		4.3	1.7
%Nominal		99.3	99.0
%RE		-0.7	-1.0
%Total Error		4.9	2.7
n		6	6

Table 13 Long Term Storage Stability Results - 5-Hydroxydantrolene -20°C

Run Date	Run Number	77 day QC Low 30.0 ng/mL	77 day QC High 4000 ng/mL
12-Apr-2013	21	31.5	~4620
		32.6	4450
		~36.4	~4710
		30.7	4460
		32.7	4480
		33.0	4300
Mean		32.8	4500
S.D.		1.96	143
%RSD		6.0	3.2
%Nominal		109.3	112.5
%RE		9.3	12.5
%Total Error		15.3	15.7
n		6	6
~ > 15%RE			

Whole Blood Stability:	Table 16: 0.5, 1, 1.5, and 2 hours on wet ice and 2 hours at ambient temperature Whole blood stability was performed for dantrolene in runs 9, 21, and 25 and met acceptance criteria at QC Low and High concentration levels following 2 hours under ambient temperature (run 9; -14.3% and -6.9%, respectively) or at QC Low, QC Mid and High concentration levels at 0.5 hours on wet ice (run 25; -12.1%, -14.4% and 2.13%, respectively). There was an observed trend in the QC Low level which failed to meet the acceptance criteria in runs 9, 21 and/or 25 for nearly each time interval assessed on wet ice. In run 25, a single pool (used to minimize variability between measurements over time) was prepared at each QC concentration level and aliquots were sampled from the pool at each stability time interval, whereas in runs 9 and 21 separate pools were spiked at QC Low and High levels for each stability interval which may have introduced bias and variability. A QC Mid concentration level, included in run 25, provided additional information with regard to the consistent change in dantrolene observed during storage on wet ice. The QC Mid concentration level, on wet ice, narrowly met the acceptance criteria at the 0.5 and 1.5 hour intervals and failed to meet the acceptance criteria at the 1.0 hour interval which is similar to the trend observed at the QC Low level. It is likely that the results observed for dantrolene (at QC Low and Mid levels) in whole blood stored on wet ice are due to poor recovery from, rather than stability in, this matrix (see Recovery, Bench Top Stability and Long Term Storage Stability). The affinity of dantrolene to bind to red blood cells likely accounts for the lack of recovery of dantrolene (irrespective of storage temperature) at QC Low and Mid levels when stored on wet ice, given the extensive partitioning of dantrolene to red blood cells and loss of analyte from centrifugation of cells during processing to plasma (as used for analysis of whole blood stability). Based on assays of whole blood and plasma, greater amounts of dantrolene are associated with red blood cells than with plasma[4],[5]. In whole blood from Beagle dog, red blood cell-to-plasma mean ratios ranged from 1.9 to 3.2 during 15 minutes to 2 hours after dantrolene administration[6]. Whole blood stability was performed for 5-hydroxydantrolene in runs 9, 21, and 25 and met the acceptance criteria at each concentration level following 2 hours under ambient temperature (run 9) and up to 1.5 hours on wet ice (run 25). As seen with dantrolene there was an observed trend at the QC Low level in runs 9 and 21 which was not observed in run 25. The discrepancy between these runs are most likely due to the execution of the assessment as there were separate pools prepared at each stability interval in runs 9 and 21, whereas run 25 utilized a single pool at each QC concentration level and aliquots were taken at each stability interval. In run 25, a QC Mid concentration level was also included and met all acceptance criteria at each time interval assessed. The affinity for 5-hydroxydantrolene to bind red blood cells is unknown, but is estimated to be analogous to dantrolene given the structural similarity of these analytes.

<table>
<tr><td>Working Internal Standard Stock Solution Stability:</td><td>Table 17:

Dantrolene-d4: Assessed at 21 and 23 hours at ambient temperature and 4, 7, and 360 days at 4°C

5-Hydroxydantrolene-d4: Assessed at 21 hours at ambient temperature and 7 days at 4°C

Stability of the working internal standard solution (containing both dantrolene-d4 and 5-hydroxydantrolene-d4) has been established for up to 21 hours of ambient temperature storage and 7 days of 4°C storage.</td></tr>
<tr><td>Conclusion:</td><td>An LC-MS/MS method for the determination of dantrolene and 5-hydroxydantrolene concentrations in human plasma with K_2EDTA has been successfully validated. The calibration range of the method is 50.0 to 25,000 ng/mL for dantrolene and 10.0 to 5,000 for 5-hydroxydantrolene using a 100 µL plasma sample aliquot. The results indicate the method is sensitive, selective, accurate, and reproducible. Dantrolene and 5-hydroxydantrolene are stable during storage, processing, and analysis.

The following sample stabilities have been determined:
<table>
<tr><td></td><td>Dantrolene</td><td>5-Hydroxydantrolene</td></tr>
<tr><td>Reinjection Reproducibility</td><td>116 injections at ambient temperature</td><td>116 injections at ambient temperature</td></tr>
<tr><td>Freeze/thaw cycle stability</td><td>3 cycles at -20°C or -70°C</td><td>3 cycles at -20°C or -70°C</td></tr>
<tr><td>Bench stop stability</td><td>25 hours at ambient temperature</td><td>20 hours at ambient temperature</td></tr>
<tr><td>Long term storage stability</td><td>188 days at -20°C, 173 days at -70°C</td><td>188 days at -20°C, 143 days at -70°C</td></tr>
<tr><td>Post-processed stability</td><td>90 hours at ambient temperature</td><td>90 hours at ambient temperature</td></tr>
<tr><td>Autosampler stability</td><td>79 hours at ambient temperature</td><td>79 hours at ambient temperature</td></tr>
</table></td></tr>
</table>

4.2.2 Sponsors response to information request sent by the agency to address hemolysis of blood samples utilized for PK analysis:

Hemolysis Assessment Methods and Results:

Appendix D

A total of 1,156 plasma samples were submitted for visual sample color grading and evaluation of hemolytic index (HI). A color scale (defining "yellow", "orange", and "red") was prepared using study samples representative of each color category (2 samples for "yellow", 2 for "orange", and 1 for "red") to ensure uniformity in color grading for the visual assessment. HI was evaluated using a Beckman Olympus analyzer with Beckman Coulter LIH reagent for determination of hemoglobin in plasma. This system (i.e. this method of analysis) was chosen as an enzymatic-assay based quantification of hemoglobin concentration was not possible due to the K_2EDTA matrix used for the bioanalytical sample processing (EDTA being known to interfere with the enzymatic assay). Based on the hemolytic index, the approximate hemoglobin concentration (mg/dL) range can be estimated (based on the manufacturer's specifications; see in-text table below) and hemolytic status was then classified using SOP-CLP-60.

In the event that the hemolytic index could not be determined by the instrumentation, hemolytic status was assessed by visual inspection and a hemolysis designation assigned per SOP-CLP-2.

Hemolytic Index (HI)	Hemoglobin Concentration (mg/dL)	Hemolysis Status
0	<50	No hemolysis
1	50-99	Slight hemolysis
2	100-199	Hemolyzed
3	200-299	Moderately hemolyzed
4	300-500	Grossly hemolyzed
6[a]	NA	NA

[a]If the Beckman Olympus analyzer returns a sample HI result of 6, the sample is reanalyzed. Following a repeat HI result of 6, the sample is inspected for visual evidence of hemolysis (in accordance with SOP-CLP-2, based on the 'Processing Hemolysis Key' specimen chart). If no visible hemolysis is present, the HI value is suppressed and a comment of "no visible hemolysis" is reported.

Results of visual sample color grading:

A total of 1,112 (of the 1,156) samples were noted as "yellow" (i.e., normal plasma color), 43 samples were noted as "orange", and one sample was noted as "red".

Hemolysis Assessment Methods and Results (continued):	Results of Hemolytic Index assessment: Hemolytic index was determined for 1,125 (of the 1,156 samples in total) samples; 1,068 samples returned a HI value of 0, 51 samples returned a HI value of 1, 5 samples a HI value of 2, and only 1 sample returned a HI of 3. For the remaining 31 samples a HI value of 6 was returned in a repeat analysis and, these samples were visually inspected in accordance with SOP-CLP-2. Each of these 31 samples was determined to have no visual evidence of hemolysis. The independent sample color grading result recorded for these 31 samples showed 28 samples to be "yellow" in color and 3 samples to be "orange". Results Summary: The data table presented in Appendix D provides a compilation of hemolytic index, plasma color, dantrolene plasma concentration, and hemolytic status. There appears to be no correlation between the measured dantrolene plasma concentration and either sample color, hemolysis status, or approximate hemoglobin concentration of samples. For the 1,125 samples with an HI value of between 0 and 3 there was a very good correlation between visual grading and hemolysis status (as determined by HI); yellow samples predominantly indicating an HI score of 0 (18 of the 1,112 yellow samples returned an HI of 1), orange samples indicating an HI score of 1 or 2 (2 orange samples had an HI of 0), and the red sample displaying an HI score of 3. Furthermore, 5 of the 61 predose samples were visually described as orange (8%), whereas only 4% of all the samples were graded as orange or red. In addition, there are no data to indicate an impact on dantrolene plasma concentration resultant from hemolysis.

Table Plasma samples from (b) (4) Research study report 1773-022 with evidence of hemolysis

Dose (mg/kg)	Study drug	Subject Identification Number	Time point(s) Post Dose[1]
1.0	Ryanodex	1001	1, 5** & 45 Minutes; 1.5 & 2 Hours
		1003	36 Hours
		1006	6, 8, 10 & 36 Hours
	Dantrium	1005	Immediately After Dose, 1 Hour
1.75	Ryanodex	2001	Immediately After Dose
		2003	10 hours
	Dantrium	2006	15 Minutes**
2.0	Ryanodex	3003	5 & 30 minutes
		3007	Immediately After Dose
2.25	Ryanodex	4002	1 Minute
		4003	1.5 & 6 Hours
		4006	1 Minute**
		4007	10 Hours
	Dantrium	4001	6 Hours
2.5	Ryanodex	5003	4 Hours**
		5006	Immediately After Dose, 15 & 45 Minutes, 2 hours
		5007	Immediately After Dose, 45 minutes
		5008	8 & 12 Hours
		6002	Immediately After Dose, 5 minutes, 10 & 12** hours
		6007	1.5 Hours
	Dantrium	5002	1 Minute, 1.5 & 10 hours
		5003	10 Hours***
		5005	Immediately After Dose, 1 Minute
		5007	30 Minutes, 1.5, 2 & 10 Hours
		6001	1 Minute, 1 Hour
		6003	Immediately After Dose
		6004	6 Hours
		6005	Immediately After Dose
		6007	Immediately After Dose, 12 Hours

[1] Unless otherwise noted, samples listed have a Hemolytic Index = 1.

** Samples with Hemolytic Index = 2

*** Samples with Hemolytic Index = 3

In addition to the above table, the sponsor complied with the Agency's request to indicate "every single" sample with regard to hemolysis status by providing about 1156 line listings. The above table is succinct representation of that information.

4.2.3 Synopsis of In Vitro binding of dantrolene to human blood.

INTRODUCTION

This study was conducted for Eagle Pharmaceuticals, Inc., to determine the *in vitro* binding of Dantrolene to human red blood cells. This nonclinical laboratory study was not intended to be conducted in accordance with the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations, 21 Code of Federal Regulations (CFR) Part 58.

MATERIALS AND METHODS

Test Article Preparation

The mixture of [^{3}H]Dantrolene sodium and Ryanodex® (dantrolene sodium for injectable suspension) was accomplished by reconstituting Ryanodex® to 50 mg/mL, then diluting to 5 mg/mL, followed by mixing [^{3}H]Dantrolene and Ryanodex® to yield a 100x concentrated stock at 15,000, 150,300, and 2,000,300 ng/mL. Preparation of 100x stocks solutions of 15,000, 150,300, and 2,000,300 ng/mL of blended [^{3}H]Dantrolene and Ryanodex® was formulated according to the table below.

Final concentration of [^{3}H]Dantrolene and Ryanodex® into whole blood

100 x stock: Dantrolene Sodium concentration (ng/mL)	100 x stock: Specific Activity (uCi/mL)	Dantrolene Sodium Concentration in Whole Blood (ng/mL)	Specific Activity in Whole Blood (uCi/mL)	Theoretical Radioactivity in Whole Blood (dpm/mL)
15,000	24.5	150	0.245	543900
150,300	25	1503	0.25	555,000
2,000,300	25	20,003	0.25	555,000

Study Procedure

Fresh male human whole blood was obtained from [redacted] (b) (4) from 4 subjects on the day of the study. Whole blood was collected in tubes pretreated with K_2-EDTA. Each whole blood collection tube was tested for hemolysis before they were used for this experiment. Briefly, a 0.5 mL aliquot was removed from each collection tube and spun at ~600 x g for 10 minutes at 4°C. The resulting supernatant (plasma) was visually inspected for color. Remaining whole blood was pooled from all four donors. Pooled whole blood was then dispensed into three 9.90 mL aliquots in separate tubes.

Final dantrolene concentrations in whole blood were 150, 1,503, and 20,003 ng/mL. Whole blood was dosed approximately 2.5 hours from the time the test article was initially put into an aqueous material. Test samples were immediately placed in a 37°C cell culture incubator and mixed by using a [redacted] (b) (4) Remaining dosed whole blood was weighted and counted by Liquid Scintillation Counting (LSC).

At 30, 60, and 120 minutes three test samples from each time point and dose concentration were removed from the incubator and centrifuged at ~600 x g for 10 minutes at 4°C. After centrifugation the test samples were visually inspected for hemolysis. Supernatant (plasma)

was removed, weighted, and the entire amount was counted by LSC. The corresponding cell pellets (Red Blood Cells; RBCs) weights were calculated by difference in weights of the centrifuge tube. Approximately 50 µl of the pellet was weighted and counted by LSC.

The RBC pellets were washed with 0.7 mL of 0.9% sodium chloride. RBC pellets were washed by gentle inversion of the centrifuge tube to disburse the RBC's into solution. Test samples were centrifuged at ~600 x g for 10 minutes at 4°C. The supernatant was weighted and counted by LSC. This process was repeated twice and after each centrifugation the samples were visually inspected for hemolysis.

After two washes, 0.5 mL of 0.9% sodium chloride, was added to the RBC pellets. Samples were placed in a dry-ice/hexane bath for one minute followed by thawing in a water bath at 37°C. Two freeze/thaw cycles were conducted on each sample. Samples were then centrifuged at ~12,000 x g for 10 minutes at 4°C. The supernatant (RBC cytosol) and pellet (cell membrane and debris) were weighted, treated and counted by Liquid Scintillation (LSC).

RESULTS

Visual analysis of each blood collect tube (pre-dose) revealed no evidence of hemolysis prior to test article addition. Dosed blood samples at all concentrations and time points were visually inspected for hemolysis; the first spin was at ~600 x g for 10 minutes at 4°C (supernatant = plasma, pellet = intact RBC), and second and third spin ~600 x g for 10 minutes at 4°C (supernatant = RBC wash, pellet = intact RBC). In all cases visual inspection did not indicate hemolysis. Samples were spun at ~600 x g for 10 minutes at 4°C where the supernatant is plasma and the pellet is intact RBCs.

Human Red Blood Cell *in vitro* Binding of Dantrolene

Mixture of [^{3}H] Dantrolene and Ryanodex® was formulated for *in vitro* binding in male human red blood cell. Incubations at 150 and 1503 ng/mL had 46% to 65% of dantrolene bound to RBCs. Test article incubations at 20,003 ng/mL had 27% to 36% dantrolene bound to RBCs. Similar dantrolene binding ranges were seen across incubations of 30, 60, and 120 minutes within the three dose dantrolene concentrations. It should be noted that for the 120 minute incubation, for one sample at 1503 ng/mL and all three samples at 20,003 ng/mL, very little pellet (cell membrane/debris) was found for the lysed sample after spinning at 12,000 x g for 10 minutes at 4°C. All other samples contained a pellet of approximately 0.1 g. Mass balance for all samples ranged from 81% to 101% recovery.

STUDY CONCLUSIONS

- For human whole blood incubated with [3H]Dantrolene and Ryanodex®, the fraction of total radioactivity in plasma *increased* with increasing dantrolene sodium concentration.
- The fraction of total dantrolene radioactivity associated with red blood cells (cytosol plus cell debris) *decreased* with increasing dantrolene sodium concentration.
- Similar dantrolene binding data was seen across incubations of 30, 60, and 120 minutes within the three dantrolene sodium treatment concentrations.
- The data indicates that in the event of blood sample hemolysis, the resultant plasma concentration of Dantrolene sodium may be elevated, due to release of Dantrolene from soluble red blood cell contents (cytosol). This observed effect would be of less significance for samples of higher Dantrolene whole blood concentrations. Incubations at 150 and 1503 ng/mL dantrolene sodium had 46% to 65% of dantrolene bound to RBCs, where as incubations at 20,003 ng/mL dantrolene sodium had 27% to 36% dantrolene bound to RBCs.
- Mass balance (total recovery of dantrolene radioactivity) ranged from 81-101%, which validates conclusions made regarding partitioning of Dantrolene between plasma and red blood cells from human whole blood.

4.2.4 Study EGL-Dantrolene-1201 a (Partially conducted by [redacted] (b) (4), Clinical Conduct Under Amendments 01 and 02)

PROTOCOL SYNOPSIS

Title	Pharmacokinetics and Safety of Dantrolene Sodium Suspension (Ryanodex®) compared to Dantrolene Sodium for Injection (Dantrium®) in Healthy Volunteers
Study Phase Study location	Single Phase 1 site in United States
Study drug Comparator	Ryanodex®: dantrolene sodium suspension for injection 0.9% Sodium Chloride Injection USP as placebo for injection Dantrium® Intravenous: dantrolene sodium for injection
Number of subjects	43 (Part 1 n=31; Part 2 n=12)
Rationale	Dantrium sodium is an approved and very effective agent for treatment and prophylaxis of malignant hyperthermia, a condition with a high mortality. Administration of the currently marketed formulation requires preparation of a large number of vials and infusion of a significant amount of fluid. Most importantly, the time and manpower required to prepare the marketed formulation can be untenable in an emergency situation. A new formulation containing dantrolene sodium in suspension (Ryanodex) has been developed by Eagle Pharmaceuticals, Inc. One vial of Ryanodex contains 250 mg of dantrolene sodium, which when reconstituted with 5 mL per vial water for injection (50 mg/mL), provides a large enough dose for a 100 kg subject receiving the recommended initial dose of 2.5 mg/kg for the [redacted] (b) (4) of malignant hyperthermia. Ryanodex would provide immediately an adequate dose of dantrolene for injection by eliminating the elaborate preparation time, allowing treatment of dantrolene with a fraction of the volume compared to the marketed product. Because the new formulation is given in less time (from less than one minute up to 5 minutes,) rather than a more prolonged injection period with the currently marketed formulation, the time to maximum blood concentration (T_{max}) is expected to be immediately following the completion of injection and much faster than achievable with the marketed product. This study is being conducted to characterize the pharmacokinetic profile and determine whether the highly concentrated nature of the new Eagle Pharmaceuticals, Inc. formulation has any effect on the safety product profile of dantrolene in conscious healthy volunteers. In Part 2 of the study the pharmacokinetic profile, safety and tolerability of single doses of Ryanodex will be compared to Dantrium in conscious healthy volunteers.
Objectives Primary: Secondary:	To characterize the single dose pharmacokinetic profile of Ryanodex in conscious healthy volunteers To determine the maximum tolerated single dose (MTD) of Ryanodex in conscious healthy volunteers To compare the single-dose pharmacokinetic profile and evaluate the safety and tolerability of Ryanodex and Dantrium in conscious healthy volunteers
Population	• Healthy male and female volunteers age 18 to 45 • Adequate peripheral veins for rapid infusion of study medications • All subjects must use adequate non-systemic contraception during the study and for one month thereafter • Females must be non-pregnant and non-lactating • Non-smokers; must abstain from alcohol for at least 2 weeks prior to study entry • Not taking any prescription or over-the-counter medications • Additional specific requirements in the body of the protocol

esign	Part 1: Ryanodex dose escalation by cohorts until an MTD is reached, as defined in the protocol, or a dose of 5 mg/kg is reached Part 2: Randomized crossover of Ryanodex versus Dantrium will be at a 2.5 mg/kg dose level or at the defined MTD dose of Ryanodex achieved in Part 1 of this study if the MTD is lower than 2.5 mg/kg .
Methodology Part 1: dose escalation:	Cohorts of 5 naïve healthy volunteer subjects each will be treated with Ryanodex (50 mg/mL) at doses of 1, 1.75, 2.5, 3.0, 4.0 and 5.0 mg/kg; One subject at each dose level will receive saline (at equivalent dose volume) as a placebo; (Total of 5 subjects per dose level [Cohort] , except initial dosing with sentinel subject). Treatment with active drug versus placebo will be administered in a randomized, double-blind fashion. The allocated drug for each subject will be administered intravenously into a running IV containing normal saline. Only one cohort will be treated on any given day. Dose-limiting toxicity (DLT) is defined as either: a. Any grade 3 (severe) or 4 (life-threatening) toxicity, or b. Increase in maximum inspiratory pressure to > -40 cm H_2O lasting >10 minutes c. Clinically significant acute changes in hepatic, renal or hematological parameters If at least **two or more subjects** in any cohort experience a dose-limiting toxicity (DLT), no additional subjects will be treated at that dose level and the previous dose level will be declared as the MTD. Optional cohort testing at a dose less than the DLT but greater than the prior MTD will be at the discretion of the Investigator and Sponsor. Additionally, intermediate doses or new dose infusion rates may be tested in separate special cohorts of up to 10 subjects, to occur between dose escalation levels during Part 1, to refine the MTD and provide additional safety and pharmacokinetic information regarding infusion rates for further dose escalations. During these special cohorts, subjects would be dosed with a single, previously utilized, dose level, but with different infusion rates. The infusion rates utilized will evaluate safety of delivery of the study drug dose over 30 seconds (as described in Part 1 dose escalation) compared with an infusion rate of a dose occurring up to 5 minutes in duration. For dosing during these special cohorts theinvestigator will be blinded to the subjects' treatments and staff other than pharmacy/nursing staff who actually administer the drug. Dosing of the cohort may be completed over a single or multiple days. A total of 12 additional naïve subjects will each receive the maximum tolerated dose of Ryanodex formulation or 2.5 mg/kg dose (whichever is lowest), and an equivalent dose of Dantrium. The two doses will be administered at least 4 days apart, to allow a period of at least 8 half-lives between administrations.
Part 2: crossover between formulations:	Ryanodex will be administered at a concentration of 50 mg/mL. The equivalent Dantrium IV dose will be administered by intravenously as per MHAUS guideline (Appendix IV) recommendations at a rapid rate of infusion. The Dantrium dose will be administered at a rate to best match the Ryanodex dose administration time period. Order of administration will be randomized. Investigator will be blinded to the subjects' treatments and staff other than pharmacy/nursing staff who actually administer the drug. This will be done by draping the subject's arm and performing the administration out of sight of all study personnel and subjects.

	In order to avoid unblinding, pharmacokinetic assessments will be performed at exactly the same time points from the start of treatment for both formulations.
Endpoints Efficacy: Pharmacokinetic: Safety:	No efficacy parameters will be studied. Pharmacokinetic parameters for dantrolene sodium and 5-hydroxydantrolene will be estimated using a non-compartmental analysis. C_{max}, T_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, $t_{½}$, Cl_p, and λ_z will be calculated, where possible. V_z, V_{dss} and AUMC will be calculated for dantrolene sodium only. The dose relationship of these pharmacokinetic parameters of dantrolene sodium and its major metabolite, 5-hydroxydantrolene, will be assessed. MTD of Ryanodex Adverse events Vital signs Handgrip strength test results Maximum inspiratory and expiratory pressure (MIP and MEP) test results Subjective status findings Clinical safety laboratory tests, including PT/PTT/INR EKG
Statistics	Analysis Dataset: All subjects treated with study drug will be included in the safety evaluation dataset. All subjects who had sufficient plasma sampling to estimate pharmacokinetic parameters will be included in the pharmacokinetics evaluations for Part 1. In Part 2, subjects who had sufficient plasma sampling to estimate pharmacokinetic parameters will be included in the pharmacokinetics summaries; only the subjects who complete both periods will be included in the relative bioavailability analysis. Patient demographics and baseline characteristics, protocol deviations, and study completion status will be tabulated. Pharmacokinetics: Part 1: Standard pharmacokinetic parameters (C_{max}, T_{max}, $AUC_{0\text{-t}}$, $AUC_{0\text{-inf}}$, $t_{½}$, Cl_p, V_z, V_{dss}, λ_z and AUMC for dantrolene and hydroxydantrolene will be tabulated for each dose group without formal statistical analysis. Part 2: Standard pharmacokinetic parameters will be tabulated by formulation for dantrolene and hydroxydantrolene. Relative bioavailability will be assessed for C_{max} and AUC following the framework of two one-sided t-tests based on the analysis of variance for crossover design. Safety: Adverse events and serious adverse events reported during Part 1 and Part 2 will be tabulated by dose and formulation. Relationship between dose level of Ryanodex and adverse event profile will be explored if data permits. Changes in clinical safety laboratory tests and vital signs will be tabulated by dose and formulation without formal inferential statistics. Findings from handgrip strength tests, subjective status evaluations, and MIP and MEP will also be tabulated by dose and formulation.

Figure **Mean (+/- 1 SD) Dantrolene Plasma Concentrations vs. Time after Administration of 1.0 (Cohort 1), 1.75 (as 30-sec Bolus-Cohorts 2, 3 & 3b; as 5-minute Infusion-Cohort 3b), or 2.0 (Cohort 3) mg/kg Ryanodex**

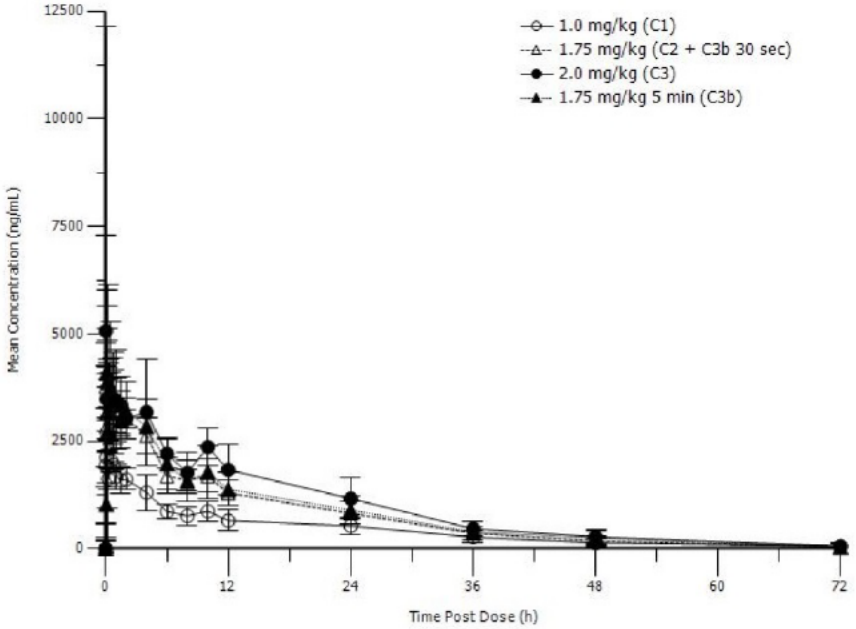


Table **Summary of Dantrolene Plasma Pharmacokinetic Parameters for 1.0, 1.75, and 2.0 mg/kg Ryanodex**

	1.0 mg/kg	1.75 mg/kg	1.75 mg/kg [Infused over 5 minutes]	2.0 mg/kg
	(N=4)	(N=9)	(N=4)	(N=2)
AUC0-24HR (hr*ug/mL)				
n	4	9	4	2
Mean	20.35	38.64	40.97	48.46
SD	5.295	7.264	11.06	15.12
AUCINF (hr*ug/mL)				
n	4	9	4	2
ean	31.24	52.71	57.46	67.78
SD	10.31	13.25	20.84	25.17
AUCLAST (hr*ug/mL)				
n	4	9	4	2
Mean	29.59	50.72	54.20	65.56
SD	9.917	12.76	19.51	25.38
CMAX (ng/mL)				
n	4	9	4	2
Mean	2710	4830	4150	6470
SD	524	1520	1010	5090
THALF (hr)				
n	4	9	4	2
Mean	15.54	12.40	12.47	11.80
SD	4.14	3.24	3.24	2.23
TMAX (hr)				
n	4	9	4	2
Mean	0.16667	0.32500	0.40417	1.00833
SD	0.23492	0.47126	0.30501	1.40243
Median	0.06667	0.16667	0.42500	1.00833
Min	0.01667	0.01667	0.10000	0.01667
Max	0.51667	1.50000	0.66667	2.500000

Data Source: (b) (4) **CSR Table 14.2.2**

Figure **Mean (+/-1SD) 5-Hydroxydantrolene Plasma Concentrations vs. Time after Administration of 1.0), (Cohort 1), 1.75 (as 30-sec Bolus-Cohorts 2, 3 & 3b; as 5-Minute Infusion-Cohort 3b), or 2.0 (Cohort 3) mg/kg Ryanodex**

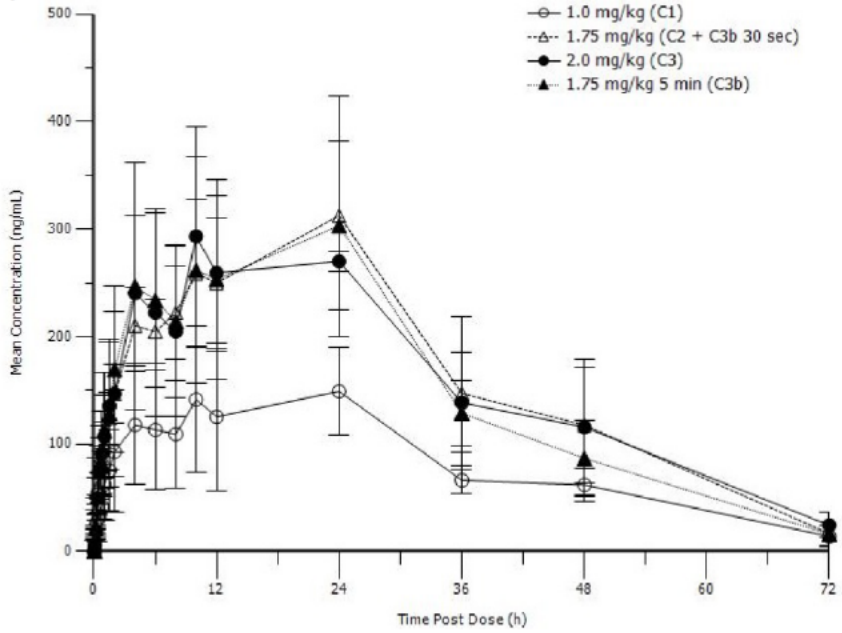


Table **: Summary of 5-Hydroxydantrolene Plasma Pharmacokinetic Parameters for 1.0, 1.75, and 2.0 mg/kg Ryanodex**

	1.0 mg/kg	1.75 mg/kg	1.75 mg/kg [Infused over 5 minutes]	2.0 mg/kg
	(N=4)	(N=9)	(N=4)	(N=2)
AUC0_24HR (hr*ug/mL)				
n	4	9	4	2
Mean	2.942	5.743	5.867	5.690
SD	1.284	1.320	1.929	1.304
AUCINF (hr*ug/mL)				
n	4	7	4	2
Mean	6.353	13.38	11.29	11.82
SD	1.023	2.814	2.016	0.8621
AUCLAST (hr*ug/mL)				
n	4	9	4	2
Mean	5.800	11.42	10.88	11.35
SD	0.8871	3.698	2.056	0.5863
CMAX (ng/mL)				
n	4	9	4	2
Mean	157	323	317	321
SD	57.3	108	91.8	63.0
THALF (hr)				
n	4	7	4	2
Mean	16.79	12.67	11.68	13.19
SD	4.02	3.43	3.48	1.39
Median	16.96	13.23	12.09	13.19
Min,	12.70	7.77	7.28	12.21
Max	20.52	17.25	15.27	14.18
TMAX (hr)				
n	4	9	4	2
Mean	20.50	19.33	19.01	17.01
SD	7.00	7.00	10.01	9.89
Median	24.00	24.00	24.00	17.01
Min,	10.00	10.00	4.00	10.02
Max	24.02	24.00	24.03	24.00

Data Source: (b) (4) CSR Table 14.2.2

In the initial protocol (Amendment 01), the dose escalation (Part 1) compared Ryanodex with Placebo, however after the initial Ryanodex dose escalations it became apparent that the planned study design was not adequate to generate useful data to determine the relative safety and tolerability of Ryanodex [to Dantrium]. This was because confounding data were produced whereby the patient was clinically stable with normal oxygen saturation, however they also presented with abnormally low (i.e. contradictory) maximum inspiratory pressure. It was therefore determined to redesign the dose escalation part whereby Ryanodex would be compared to Dantrium at all dose levels. In addition, the dose limiting toxicity (DLT) criteria needed to be changed to demonstrate safe escalation of dose. See results of amended clinical study next.

4.2.5 Study EGL-Dantrolene-1201 (Conducted by Comprehensive Clinical Development, Tacoma, WA; Bioanalysis conducted by (b) (4) Amendments 3 and 4)

2. SYNOPSIS

Sponsor:	Eagle Pharmaceuticals, Inc.	
Name of Finished Product:	Dantrolene Sodium Suspension (Ryanodex®)	
Name of Active Ingredient:	Dantrolene Sodium	
Study Title: Pharmacokinetics and Safety of Dantrolene Sodium Suspension (Ryanodex®) compared to Dantrolene Sodium for Injection (Dantrium® Intravenous) in Healthy Volunteers [Amendment 03 (06-Dec-2012),Amendment 04 (30-Jan-2013)]		
Investigator(s): Jon L. Ruckle, MD, Comprehensive Clinical Development, Inc., 3615 Pacific Ave. Tacoma, WA98418		
Study Center: Comprehensive Clinical Development, Inc., 3615 Pacific Ave. Tacoma, WA98418		
Publication: None		
Study Period: 18-Dec-2012 to24-Feb-2013		Study Phase:1
Initiation Date (first subject randomized): 20-Dec-2012		
Completion Date (last subject completed): 24-Feb-2013		

Study Objectives:

Primary:

To characterize the single dose PK profile of Ryanodex in conscious healthy volunteers

Secondary:

To determine the MTD of Ryanodex and/or Dantrium for comparison in conscious healthy volunteers

To compare the single-dose pharmacokinetic profile and evaluate the safety and tolerability of Ryanodex and Dantrium in conscious healthy volunteers

Methodology:

Study Design:

This study was conducted in two parts at a single study center(CCD):

Part 1: Ryanodex and Dantrium dose escalation by dose levels until an MTD (as defined in the protocol) or the dose of 2.5 mg/kg was reached.

Part 2:Randomized crossover of Ryanodex versus Dantrium at the 2.5 mg/kg dose level or at the defined MTD of Ryanodex and/or Dantrium achieved in Part 1 of this study if the MTD was lower than 2.5 mg/kg.

Study Duration:

The study duration was approximately 9 weeks from screening of the first subject group until last follow-up visit of the last dose group.

Subject Participation:

The duration of individual subject participation from start of screening through final follow-up was approximately3-4weeks. Pre-study screening for eligibility occurred up to30 days before the first dose of study drug. Subjects were admitted to the Clinical Research Unit (CRU) the day before dosing (Day-1).

Ryanodex or Dantrium was administered in the morning after an overnight fast. Subjects remained in the CRU for 72 hours after dosing for safety and PK assessments. A follow-up telephone call was made to each subject at 1-2 weeks after dosing.

Plasma PK: Blood samples for plasma concentrations of dantrolene and its metabolite, 5-hydroxydantrolene, were collected from subjects in Parts 1 and 2 at just prior to dosing (-45 to 0 min), immediately after dosing, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes; and 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after dosing.

Dose Escalation:

Part 1 (Single dose escalation):

An initial cohort of 6 healthy male subjects was administered a single intravenous (IV) dose of 1.0 mg/kg of Ryanodex (50 mg/mL) or Dantrium (0.33 mg/mL). Three (3) subjects received Ryanodex and 3 subject received Dantrium. Data on the safety and tolerability of this dose group was reviewed, as well as from the 1.0 mg/kg and 1.75 mg/kg dose groups completed in the previous (b) (4) study, and a decision was made by the Sponsor to proceed to the next dose level without enrolling further subjects at the 1.0 mg/kg dose. Subsequent treatment groups of 4 healthy male subjects were treated with either Ryanodex (50 mg/mL) or Dantrium (0.33 mg/mL) at doses of 1.75, 2.0, 2.25, and 2.5 mg/kg. At these dose levels, one treatment group of 4 subjects received Ryanodex, and a separate treatment group of 4 subjects received Dantrium at each of the dose levels, unless dose limiting toxicities are discovered. In all cohorts, subjects were randomized to either study treatment. Equal numbers of subjects for each treatment were studied on a single day in a double blind manner.

Treatment with active drug was administered in a randomized, double-blind fashion. The allocated drug for each subject was administered as a single IV infusion.

The following DLT criteria were used in defining the MTD and played a role in ensuring the safe continuation to the next dose level (in the dose escalation part).

Study DLT was defined, using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0 criteria, as one or more of the following:

a. Any grade 3 or 4 toxicity posing a danger to the wellbeing of the subject, or
b. Type 2 respiratory failure determined by ABGs, requiring mechanical ventilation, or
c. Clinically significant acute changes in hepatic, renal or hematological parameters which pose a danger to the wellbeing of the subject.

If two or more subjects in either treatment group experienced a DLT, no additional subjects would be treated at that dose level and the previous dose level would be declared as the MTD. Optional testing of both treatment groups at a dose less than the DLT but greater than the prior MTD would be at the discretion of the Investigator and Sponsor

Part 2: (Crossover dose between Ryanodex and Dantrium treatments)

Fifteen (15) healthy male and female subjects received 2.5 mg/kg dose of Ryanodex and an equivalent dose of Dantrium in a crossover design. The two doses were administered at least 4 days apart, to allow a period of at least 8 half-lives between administrations.

Number of Subjects (planned and analyzed): Up to52subjects were planned; 46subjects were enrolled and received at least one dose of study drug and were analyzed for safety; all randomized subjects were included in the analyses of pharmacokinetic variables.

Diagnosis and Main Criteria for Inclusion:

- Healthy male volunteers (Part 1) and healthy male and female volunteers (Part 2); age 18 to 45
- Female and male subjects with body mass index (BMI) between18-30 kg/m^2 inclusive, minimum body weight 50 kg; maximum body weight 100 kg
- Adequate peripheral veins for rapid infusion of study medications
- Adequate radial artery for arterial catheterization
- Use of an adequate contraception during the study and for one month thereafter
- Non-pregnant and non-lactating, if female
- Non-smokers; must abstain from alcohol for at least 1 week prior to study entry
- Not taking any prescription within 14 days or over-the-counter medications within 7 days prior to study drug administration

Additional specific requirements can be found in the protocol (see Appendix 16.1.1, Amendments 03 and 04).

Test Product, Dose and Mode of Administration, Lot Number:

Test Product: Ryanodex

Doses: Single IV administration in the morning after an overnight fast. Dose escalation was 1.0, 1.75, 2.0, 2.25 and 2.5 mg/kg.

Duration and Mode of Administration: Single one (1) minute infusion.

Lot Number: B120167

Reference Product, Dose and Mode of Administration, Lot Number:

Reference Product: Dantrolene Sodium for Injection (Dantrium® Intravenous)

Doses: Single IV administration in the morning after an overnight fast. Dose escalation was 1.0, 1.75, 2.0, 2.25 and 2.5 mg/kg.

Duration and Mode of Administration: Single infusion at a rate of 50 mL per minute for the required dose volume.

Lot Number:221157

Criteria for Evaluation:

Safety:

Safety was assessed by physical examinations, vital signs, pulse oximetry, arterial line blood pressure and ABG monitoring, 12-lead electrocardiograms (ECG) with 36 hour telemetry, grip strength testing, calf raises, head lift, stair climb, maximum inspiratory and expiratory pressure (MIP and MEP), Likert Scales, and clinical laboratory tests (hematology, chemistry, coagulation, and urinalysis). Adverse events (AEs) were monitored and recorded in the case report forms (CRFs) throughout the study.

Pharmacokinetics:

Standard PK parameters were derived for both dantrolene and 5-hydroxydantrolene. In addition to calculation of the actual parameters at each dose level, dose normalized parameters were calculated for the dose escalating portion of the study.

Statistical Methods:

The dose escalation portion of the study (Part 1) used a standard 4 plus 4 active treatment dose escalation format. The sample size for Part 2 of the study was chosen empirically. Please refer to the Statistical Analysis Plan (Appendix 16.1.9) for sensitivity.

Analysis Dataset:

All subjects treated with study drug were included in the safety evaluation dataset. All subjects who had sufficient plasma sampling to estimate PK parameters were included in the PK evaluations for Part 1. In Part 2, subjects who had sufficient plasma sampling to estimate PK parameters were included in the PK summaries; only the subjects who completed both periods were included in the relative bioavailability analysis.

Subject demographics and baseline characteristics, protocol deviations, and study completion status were tabulated.

Pharmacokinetics:

Part 1: Standard PK parameters (C_{max}, T_{max}, AUC_{0-24}, AUC_{0-last}, AUC_{0-inf}, $t_{½}$, Cl_p, V_z, V_{dss}, λ_z, and AUMC) for dantrolene and 5-hydroxydantrolene were tabulated for each dose group without formal statistical analysis.

Part 2: Standard PK parameters were tabulated by formulation for dantrolene and 5-hydroxydantrolene. Relative bioavailability was assessed for C_{max} and AUC following the framework of two one-sided t-tests based on the analysis of variance for crossover design.

Safety:

Adverse events and serious adverse events reported during Part 1 and Part 2 were tabulated by dose and formulation. Relationship between dantrolene dose level and adverse event profile was explored if data permitted.

Changes in clinical safety laboratory tests, including ABG and vital signs were tabulated by dose and formulation without formal inferential statistics.

RESULTS AND CONCLUSIONS

Subject Disposition:

Forty-six (46) subjects were enrolled in the study and all completed the study as required by the protocol. Dantrolene doses up through 2.5 mg/kg were safely administered in conscious, healthy male volunteers in Part 1. Seven (7) subjects of the 2.5 mg/kg dose group in Part 1 voluntarily crossed-over to receive the other study treatment (double blinded) in Part 2 of the study. An additional 6 female subjects and 2 male subjects enrolled in the crossover portion of the study, for a total of 15 subjects in Part 2.

Pharmacokinetic Results:

Part 1: Dose proportionality

Maximum plasma concentrations for Dantrolene were achieved by approximately 1-4 minutes postdose for Ryanodex treated subjects and immediately after dosing for Dantrium treated subjects and ranged on average from 2,713 ng/mL to 8,978 ng/ml for Ryanodex and 2,560 ng/mL to 5,715ng/mL for Dantrium over the dose range tested. The highest individual subject Cmax values recorded for Ryanodex and Dantrium were 20,200 ng/mL and 7,940 ng/mL respectively. Plasma concentrations for the metabolite, 5-hydroxydantrolene, followed a similar but delayed pattern. Maximum concentrations for 5-hydroxydantrolene were achieved by approximately 10 hours to 24 hours (median) for Ryanodex and 9 to 24 hours (median) for Dantrium and ranged, on average, from 254.0 ng/mL to 640.3 ng/mL for

Ryanodex and 189.7 ng/mL to 644.2 ng/mL for Dantrium over the 1.0 mg/kg to 2.5 mg/kg dose range tested.

For dantrolene, mean $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ increases were evident with increasing doses for both Ryanodex and Dantrium; however there was no apparent difference between the two treatments. Mean dantrolene $AUC_{0\text{-inf}}$ estimates ranged from 18.04 hr*ug/mL to 77.272hr*ug/mL for Ryanodex and 17.87 hr*ug/mL to 71.61 hr*ug/mL for Dantrium over the 1.0 mg/kg to 2.5 mg/kg dose range. A similar result was seen for the metabolite, 5-hydroxydantrolene. The lack of difference between Ryanodex and Dantrium in $AUC_{0\text{-inf}}$ is most likely due to the rapid clearing of the both drugs over the 72 hour PK sampling period.

The mean $t_{1/2}$ for both dantrolene and 5-hydroxydantrolene was independent of dose or treatment administered. For dantrolene, $t_{1/2}$ ranged from 8.52 (2.25 mg/kg dose) to 10.75 (2.5 mg/kg dose) hours for Ryanodex and 8.68 (2.0 mg/kg dose) to 11.40 (1.0 mg/kg dose) hours for Dantrium. For 5-hydroxydantrolene, $t_{1/2}$ ranged from 9.69 (1.75 mg/kg dose) to 13.19 (1.0 mg/kg dose) for Ryanodex and 9.33 (2.25 mg/kg dose) to14.11 (1.0 mg/kg dose) for Dantrium.

The relative bioavailability assessment demonstrated that at the 2.5 mg/kg dose dantrolene $AUC_{0\text{-inf}}$ and C_{max} were 6% and 44% higher for Ryanodex as compared to Dantrium based on the geometric mean ratio (GMR).The 90% CIs demonstrated that the two 2.5 mg/kg treatments were equivalent based on $AUC_{0\text{-inf}}$. Significant differences between Ryanodex and Dantrium were evident for C_{max} as the 90% CI was 1.18-1.75, suggesting that the two treatments were not equivalent at peak concentration after administration. For 5-hydroxydantrolene, $AUC_{0\text{-inf}}$ and C_{max} estimates were comparable between the two treatments as the 90% CI were within the 80-125% equivalence limits. This difference in $C_{max,}$ however, can be expected based on the different rates of administration. Higher C_{max} values for Ryanodex (relative to Dantrium) are consistent with the more rapid administration rate of Ryanodex.

Dose proportionality was evident for both Ryanodex and Dantrium at doses of 1.0 mg/kg to 2.5 mg/kg for dantrolene C_{max} and 5-hydroxydantrolene $C_{max,}$ and $AUC_{0\text{-inf}}$ as the 95% confidence intervals for the slope term, β, contains 1.A slight deviation from dose proportionality is evident for dantrolene $AUC_{0\text{-inf}}$ for both treatments as the lower limit of the 95% confidence intervals were greater than 1, indicating dantrolene exposure was slightly greater than proportional to the dose administered.

Based on graphical comparisons, the concentrations of dantrolene and 5-hydroxydantrolene appear to be slightly higher in females when compared to males. This may be due to the small sample size of the tested population.

Safety Results:

There were no severe adverse events, serious adverse events (SAEs) or deaths in the study. No subjects were withdrawn from the study due to an adverse event.

A dose of 2.5 mg/kg was safely reached in Part 1 of the study for both Dantrium and Ryanodex in conscious healthy subjects. There were no dose limiting toxicities reported, however, reports from the Principal Investigator (PI) to the IRB indicated subjects would have been unlikely to tolerate higher doses. This is consistent with previous data on the intravenous administration of dantrolene in healthy volunteers where dose limiting side effects were observed at 3.0 mg/kg (Kentsch 1991).

Treatment emergent adverse events increased in frequency with increasing doses in the study, but did not differ in frequency between the two formulations (Ryanodex and Dantrium). Subjects receiving Ryanodex were more likely to report immediate adverse events of flushing, dystonia, and dysphagia than those receiving Dantrium. This is most likely due to the earlier and higher plasma concentrations seen with Ryanodex. Subjects receiving Dantrium were more likely to experience headache.

No Grade 3 or 4 toxicity by NCI CTCAE (Version 4.0) criteria was observed in any of the subjects studied. No respiratory failure as defined by ABG results occurred. No hepatic or renal changes that posed a danger to

subjects' well-being were observed during the study. There were no severe or serious adverse events, no DLTs, and no subject discontinued the study due to adverse events. All events that occurred during the study were considered mild or moderate and all resolved without sequelae.

Creatine phosphokinase (CPK) declined in most subjects during the study. This decline was expected given the pharmacological action of dantrolene sodium (a skeletal muscle relaxant) and the lack of activity while confined in the CRU. This decline in CPK was not considered clinically significant. Other mean changes from baseline in hematology, chemistry, coagulation, ABG and urinalysis values or differences between treatment groups were generally small and not clinically meaningful.

There were no clinically meaningful changes from baseline in vital signs or ECG assessments during the study.

In all dose groups, hand grip strength declined after dosing. In general, the decline in hand grip strength was more pronounced and occurred more rapidly in the Ryanodex treated subjects (versus those receiving Dantrium) at the 1.0, 1.75, 2.0 and 2.25 mg/kg treatment groups. In the 2.5 mg/kg treatment group, the decline in hand grip strength both in amount and duration was very similar between the two treatment groups. This may indicate a more rapid distribution of dantrolene for Ryanodex at this dose.

Gender differences were evaluated in the 2.5 mg/kg dosing group. While the number of male and female subjects reporting at least one AE was similar between treatment groups, males were more likely to report multiple events and only male subjects reported dysphonia and flushing. There were no clinically meaningful differences in safety assessments by gender. The reasons for the gender differences observed are unknown but may be an artifact of the small sample size.

Finally, although a transiently higher C_{max} and shorter T_{max} were observed for Ryanodex (an expected consequence of the faster dantrolene sodium administration rate achievable with the Ryanodex formulation) this did not translate into any difference in safety between the two study treatments. Additionally, AEs observed during study conduct were similar to those reported in published data in healthy volunteers.

Conclusions:

Ryanodex is equivalent to Dantrium (when given as a single dose as a rapid IV push from 1.0 – 2.5 mg/kg) in all pharmacokinetic assessments with the exception of a transiently higher and quicker maximum plasma concentration for Ryanodex. Ryanodex and Dantrium were both safe and well tolerated across the range of doses in this study up to and including the 2.5 mg/kg crossover dose. Ryanodex is equivalent to Dantrium in study measures of safety and tolerability. The differences in maximum plasma concentration do not significantly affect the safety or tolerability of Ryanodex.

Figure **Mean Ryanodex Dantrolene Plasma Concentration vs. Time by Dose Group (0-2hrs)**

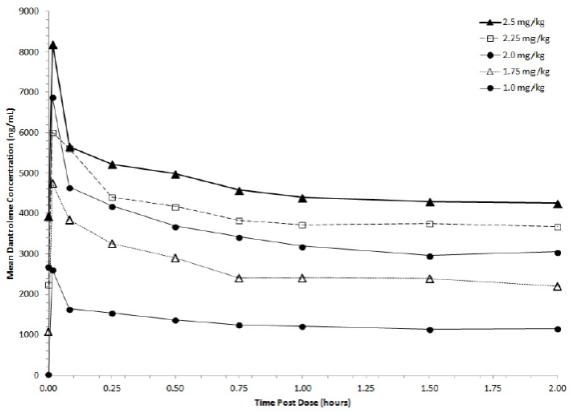
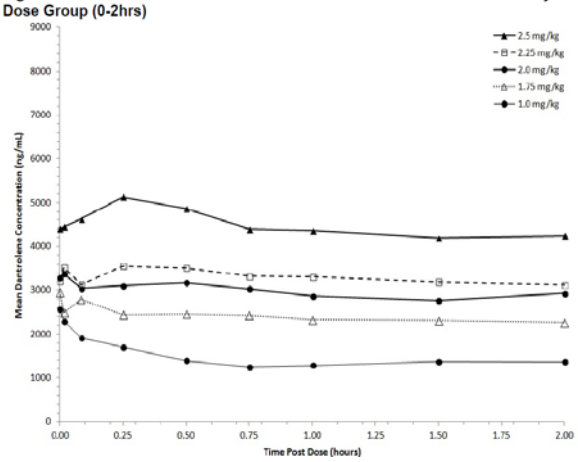


Figure **Mean Dantrium Dantrolene Plasma Concentration vs. Time by Dose Group (0-2hrs)**



Dose proportionality for dantrolene and 5-hydroxydantrolene within a treatment group was evaluated using Cmax and AUC0-inf following drug administration. A power model was fit to describe the relationship between Y (Cmax and AUC0-inf) and X (dose) using the least-squares linear regression model [log(Y) = log(α) + β log(X) where the relationship between X and Y are expressed as Y = α X β]. From each model, the α and β parameters are presented along with the 95% confidence intervals for β. Statistical summaries for the analysis of dose proportionality, presenting the results of the power model, are shown for Cmax and AUC0-inf in the table below.

Table 2.7.2-13: Dose Proportionality Evaluation for Dantrolene and 5-Hydroxydantrolene

Treatment	Dependent Variable	alpha	Slope Parameter (beta) Estimate +/- SE	95% Confidence Interval
Dantrolene				
Ryanodex	$\log(AUC_{0\text{-inf}})$	14.7	1.69 +/- 0.212	(1.25 ,2.12)
	$\log(C_{max})$	2480	1.30 +/- 0.294	(0.702 ,1.90)
Dantrium	$\log(AUC_{0\text{-inf}})$	15.9	1.51 +/- 0.231	(1.04 ,1.98)
	$\log(C_{max})$	2170	0.937 +/- 0.194	(0.540 ,1.33)
5-Hydroxydantrolene				
Ryanodex	$\log(AUC_{0\text{-inf}})$	7.94	1.07 +/- 0.188	(0.683 ,1.45)
	$\log(C_{max})$	264	0.942 +/- 0.230	(0.470 ,1.41)
Dantrium	$\log(AUC_{0\text{-inf}})$	5.80	1.34 +/- 0.167	(0.994 ,1.68)
	$\log(C_{max})$	192	1.27 +/- 0.217	(0.823 ,1.71)

Dose proportionality is evident for both Ryanodex and Dantrium at doses of 1.0 mg/kg to 2.5 mg/kg for dantrolene Cmax and 5-hydroxydantrolene Cmax and AUC0-inf as the 95% confidence intervals for the slope term, β, contains 1. A slight deviation from dose proportionality is evident for dantrolene AUC0-inf for both Ryanodex and Dantrium as the lower limit of the 95% confidence intervals were greater than 1, indicating dantrolene exposure was slightly greater than proportional.

Part 2: Dantrolene PK following administration of 2.5 mg/kg of Ryanodex and Dantrium.

Subjects that participated in Parts (periods) 1 and 2 comprised the 2.5 mg/kg of Ryanodex (N=15) or Dantrium (N=16) crossover treatment group. At 1 minute post end of dose, mean (±SD) dantrolene concentration with Ryanodex was significantly higher than that of Dantrium (8186 ± 3949 vs. 4445 ± 1618 ng/mL). Concentrations for Ryanodex remained higher than those for Dantrium until approximately 15 minutes post dose (see Figures below) after which point concentrations for both study drugs were comparable. At all other time points the plasma concentrations were, by visual check, comparable for both study drugs and well within 1 standard deviation. Following administration of 2.5 mg/kg, mean maximum concentrations for dantrolene were achieved by approximately 1.2 minutes (0.02 hours=median tmax) for Ryanodex and 15

minutes (median tmax) for Dantrium (see figure below). Mean Cmax was 8978 ng/mL for Ryanodex and 5715 ng/mL for Dantrium. Higher Cmax values for Ryanodex (relative to Dantrium) are consistent with the more rapid (dantrolene) administration rate of Ryanodex. The individual Cmax for subjects in Part 1 and 2 are presented in the Table below. In the majority of subjects (10 out of 16), the Cmax estimates for Ryanodex are higher than those observed for Dantrium.

Table Dantrolene Plasma C_{max} (ng/mL) values from Subjects administered 2.5 mg/kg Ryanodex or Dantrium

Subject identification number	Ryanodex (n=15)	Dantrium (n=16)
Parts 1 and 2		
5001	9660	5220
5002	*NA*	7130
5003	5840	4890
5004	16300	6040
5005	10200	4970
5006	5110	3840
5007	7540	4740
5008	6240	6560
Part 2 only		
6001*	12100	7110
6002*	12100	7320
6003*	8460	4500
6004	4450	6300
6005*	7530	7940
6006*	20200	5240
6007*	5470	5900
6008	3470	3750
MEAN	**8978**	**5716**

***Female (all other subjects are male).**
Data Source: Listing 16.2.5.4 and Table 14.2.2

Relative Bioavailability: For intravenous products bioavailability is 100%. Since the treatments, Ryanodex and Dantrium, were administered in a cross-over fashion in healthy volunteers, comparison of bioavailability was possible. The sponsor evaluated relative bioavailability of Dantrolene and 5-hydroxydantrolene, based on Cmax and AUC0-inf in all subjects that received both treatments of 2.5 mg/kg Ryanodex and Dantrium in the part 2 of the study. A summary statistical analysis is presented in the table below.

For dantrolene, the 90% confidence intervals (CI) demonstrated that the two treatments were equivalent for AUC0-inf (using a 90% CI criteria of 80-125%). Significant differences between Ryanodex and Dantrium were evident for Cmax as the 90% CI range was 1.18-1.75. **This is likely a direct result of dosage form (i.e. the higher dantrolene concentration in the Ryanodex formulation, versus Dantrium) and the difference in dantrolene administration rate (mg/min).** The relative bioavailability results demonstrate that $AUC_{0\text{-inf}}$ and C_{max} were 6% and 44% higher for Ryanodex as compared to Dantrium based on the GMR.

Table 2.7.2-12: Relative Bioavailability Results for Dantrolene and 5-Hydroxydantrolene

	Ryanodex			Dantrium			Ryanodex/Dantrium	
Analyte/Parameter	N	GM	95% CI	N	GM	95% CI	GMR	90% CI
Dantrolene								
$AUC_{0\text{-}inf}$ (hr*ug/mL)	15	74.5	(63.0,88.1)	15	70.3	(60.5,81.7)	1.06	(0.99,1.14)
C_{max} (ng/mL)	15	7960	(6090,10400)	15	5530	(4940,6180)	1.44	(1.18,1.75)
5-hydroxydantrolene								
$AUC_{0\text{-}inf}$ (hr*ug/mL)	14[a]	20.2	(17.0,24.1)	14[a]	19.2	(16.2,22.7)	1.05	(0.99,1.13)
C_{max} (ng/mL)	15	593	(477,737)	15	602	(483,749)	0.99	(0.90,1.08)

GM=Geometric Mean; GMR= Geometric Mean Ratio
A linear mixed effects model is used with fixed effects terms for treatment and period. A log transformation is applied to the $AUC_{0\text{-}inf}$ and C_{max} data. Back-transformed summary statistics and inferential results are reported for pharmacokinetic parameters.
[a]$AUC_{0\text{-}inf}$ for 5-hydroxydantrolene was only estimable in N=14 subjects; this exposure parameter was not calculable for female subject 6002, based on available data for this analyte.

For 5-hydroxydantrolene, AUC0-inf and Cmax estimates were comparable between Ryanodex and Dantrium as the 90% CI were within the 80-125% equivalence criteria.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SRIKANTH C NALLANI
06/27/2014

YUN XU
06/27/2014

ONDQA BIOPHARMACEUTICS REVIEW

NDA#:	**205-579/S-000**
Submission Date:	1/21/2014, 4/1/2014, 4/17/14, 5/2/14, 6/10/14
Drug Name:	Ryanodex® (dantrolene sodium)
Formulation:	lyophilized powder for injection
Strength:	Reconstituted to 250 mg/5 mL
Applicant:	Eagle Pharmaceuticals
Reviewer:	John Duan, Ph.D.
Submission Type:	Original NDA 505(b)(2)

Ryanodex® (dantrolene sodium) is a lyophilized product which is reconstituted at the time of use with 5 mL of Sterile Water for Injection.

COMMENTS

1. The Agency and the Applicant have reached an agreement on the in vitro dissolution method and acceptance criterion as shown below. The NDA has been updated based on the agreement.

Apparatus:	Apparatus II (Paddles)
Paddle Speed:	50 RPM (and 250 RPM for infinity)
Temperature:	37.0 ± 0.5°C
Medium:	0.5% BAC (benzalkonium chloride)
Volume:	900 mL
Volume Pulled:	10 mL
Filter:	0.45 µm Nylon Filter

   Acceptance criterion: Q = (b)(4)% at 1 min

2. The dissolution study conducted in human plasma provides evidence (from an in vitro perspective) to support a rapid dissolution of Ryanodex upon exposure to human plasma at a dose of 175 mg.

RECOMMENDATION

An approval is recommended from the ONDQA Biopharmaceutics perspective.

_____ _____

John Duan, Ph.D. Date
Reviewer
ONDQA Biopharmaceutics

_____ _____

Tapash Ghosh, Ph.D. Date
Team Leader
ONDQA Biopharmaceutics

cc: NDA 205-579 *DARRTS*

BIOPHARMACEUTICS EVALUATION

1. Introduction

Ryanodex® (dantrolene sodium) is a lyophilized product which is reconstituted at the time of use with 5 mL of Sterile Water for Injection. Each sterile single-use vial contains 250 mg of dantrolene sodium (b) (4).

2. The compositions of the drug products

The drug product contains 250 mg of dantrolene sodium (b) (4), 125 mg of mannitol, 4 mg of povidone (b) (4) and 25 mg of polysorbate 80 per vial. The unit composition of Ryanodex® (dantrolene sodium) powder for injection is shown in the following table.

Table: Composition of Ryanodex® (Dantrolene Sodium) powder for injection

Ingredient	Quality Standard	Amount per Vial (mg)	Function
Dantrolene sodium (b) (4) *	USP	250	Active Ingredient
Mannitol	USP/EP	125	(b) (4)
Povidone (b) (4)	USP	4	
Polysorbate 80	NF/EP	25	
Hydrochloric Acid	NF	pH adjustment	
Sodium Hydroxide (b) (4)	NF	pH adjustment	
Water for Injection	USP	(b) (4)	

* (b) (4)

Reviewer's Comments: *The formulation contains* (b) (4)

3. Dissolution of the drug product

The following dissolution method and acceptance criterion were proposed:

Apparatus:	Apparatus II (Paddles)
Paddle Speed:	50 RPM (and 250 RPM for infinity)
Temperature:	37.0 ± 0.5°C
Medium:	0.5% BAC (benzalkonium chloride)
Volume:	900 mL
Volume Pulled:	10 mL
Pull Times:	(b) (4)
Filter:	0.45 µm Nylon Filter

Acceptance criterion: Q* (% concentration relative to concentration (b) (4) min infinity) (b) (4)% in 1 minute

Reviewer's Comments: *An acceptance criterion was set at 1 minute due to the rapid dissolution, indicating the* (b) (4) *in the dissolution medium might be too high. An information request (IR) was sent in this regard. See sections below.*

1) Justification of the dissolution conditions

The studies for selection of dissolution media began with performing dissolution testing in four different media, including (b) (4) Samples were analyzed using a UV spectrophotometer. The dissolution parameters are shown below:

Apparatus:	USP II (Paddles)
Paddle Speed:	50 RPM (and 250 RPM for infinity)
Temperature:	37.0 ± 0.5°C
Volume:	900 mL
Vol. Pulled:	(b) (4)
Pull Times:	(b) (4)
Filter:	0.45µm Nylon Filter
Medium:	(b) (4)
Amt added:	(b) (4)
Analysis:	(b) (4)

In the dissolution vessels, visible precipitation was noted for (b) (4) itself since particulates were observed in the media in the absence of Dantrolene Sodium Suspension.

(b) (4)

10 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

---

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JOHN Z DUAN
06/23/2014

TAPASH K GHOSH
06/24/2014