Survival Efficacy Evaluation of 3,4-Diaminopyridine Phosphate in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1

Final Report

January 13, 2023

for

Study Number B06188

To

SPONSOR:
Defense Threat Reduction Agency
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by

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SIGNATURES

[redacted] 18 Jan 2023

Study Director Date

[redacted] 1/18/2023

Manager, High Hazard Containment – Chemistry Date

GLP COMPLIANCE STATEMENT

This study was conducted at the Battelle Biomedical Research Center (BBRC) using practices consistent with, but not in strict accordance with, 21 CFR Part 58, Good Laboratory Practice (GLP) Regulations. Facility standard operating procedures (SOPs) and practices were followed.

SUMMARY

The purpose of this study was to evaluate the capacity of 3,4-diaminopyridine phosphate (amifampridine phosphate drug substance; 3,4-DAPP) treatment to promote survival in cynomolgus monkeys systemically intoxicated with botulinum neurotoxin serotype A1 (BoNT/A1). To accomplish this, male cynomolgus monkeys (of Mauritius origin) were systemically intoxicated with BoNT/A1 by a single intramuscular (IM) injection of a BoNT/A1 dosing solution into the right calf muscle at a challenge level of 2.5x the BoNT/A1 median lethal dose (LD_{50}) for IM intoxication in cynomolgus monkeys. Following challenge, animals were observed for toxic signs commonly associated with botulism (e.g., ptosis, muscular weakness, respiratory distress, oral/nasal discharge). Clinical severity scoring was performed based on the presence and/or severity of select toxic signs. A clinical severity score of 1 or greater served as a trigger to initiate subcutaneous (SC) treatments. Animals received SC treatment with either vehicle (saline), or 4.12 mg/kg of 3,4-DAPP. After initial trigger and treatment, repeat treatments were performed at approximately 2-hour intervals thereafter. Endpoints used to evaluate potential reversal of BoNT/A1-induced toxicity with 3,4-DAPP treatment included clinical observations and associated clinical severity scores. Time-to-death calculations were performed based on mortality outcomes.

Following IM administration of BoNT/A1 at a challenge level of 2.5x LD_{50}, with the exception of occasional non-botulinum stool-related issues (soft stool, diarrhea, etc.) commonly seen with this species, all animals were observed to remain in clinically normal status for approximately 54 hours. The time-to-first observation of toxicity following challenge ranged from 53 hours and 14 minutes to 67 hours and 27 minutes. Leading signs of intoxication (greater than 50 instances) included lethargy, hunched posture, labored breathing, ptsosis, ataxia, decreased respirations, and gasping. The number of abnormal observations increased over time as disease progressed. Other abnormal clinical signs observed following challenge were oral discharge, shallow respirations, nasal discharge, forced abdominal respirations, increased respirations, prostrate posture, and death. All abnormal observations were considered specifically attributed to administration of the challenge agent (BoNT/A1). In general, for most of the time points starting Day 3 at 2000 through the last time point when appropriate comparisons could be made (Day 5 at 0600) the severity of clinical observations following BoNT/A1 challenge was significantly greater in the saline treated animals versus the animals administered 4.12 mg/kg of 3,4-DAPP. ($p = 0.0056$ to 0.0484). Within the same time frame, the frequency of some of the observations associated with intoxication (lethargy, hunched posture, decreased respirations, or gasping) were significantly greater in the saline treated animals versus the animals administered 4.12 mg/kg of 3.4-DAPP ($p = 0.0005$ to 0.0386). At the dosing frequency interval employed in this study (every 2 hours), SC treatment with 3,4-DAPP mg/kg did not completely prevent disease progression after initial presentation of toxicity as all animals on study succumbed to challenge prior to scheduled euthanasia at 96 ± 2-hours following initiation of treatment. Subjective evidence of acute toxicity reversal following 3,4-DAPP dosing was found to be supported by a general plateau or slight reduction in clinical severity scores at various times during the post-challenge observation and treatment period. Partial prevention, reversal, or reduction of clinical severity in some animals following 3,4-DAPP dosing was evidenced by the absence of oral/nasal discharge in most animals at all time points, resolution of ptosis for an extended period of time post-challenge, and lack of increase in the severity of respiratory-related signs.

The time to death post challenge after SC administration of 4.12 mg/kg 3,4-DAPP every 2 hours ranged from 94.30 hours (h) to 135.65h (median: 115.71 ± 13.95h), and for control animals receiving saline ranged from 85.23h to 111.2h (median: 94.38 ± 8.94 h). The time to death post trigger after initiation of SC administration of 4.12 mg/kg 3,4-DAPP every 2 hours ranged from 30.75h to 73.90h (median: 52.20 ±16.43h), and for control animals receiving saline every 2 hours ranged from 26.13h to 52.13h (median 35.38 ±8.64h). The median life extension attributed to 3,4-DAPP (beyond untreated animals) post challenge and post trigger was 21.33h and 16.82h, respectively. The study indicates post-symptomatic administration of 3,4-DAPP treatment promotes survival in cynomolgus monkeys systemically intoxicated with BoNT/A1.

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1 INTRODUCTION

The purpose of this study was to evaluate the therapeutic capacity of 3,4-DAPP to enhance survival in cynomolgus monkeys systemically intoxicated with botulinum neurotoxin serotype A1 (BoNT/A1).

This study was conducted at the Battelle Biomedical Research Center (BBRC) under the direction of [redacted] The study was initiated on March 29, 2022, with the signing of the protocol. The in-life portion of the study began on April 11, 2022, with the initiation of BoNT/A1 challenge and was concluded on April 16, 2022 when the final animal succumbed to challenge.

Intramuscular challenge administration of BoNT/A1 was performed on a single challenge day. Post-challenge subcutaneous treatments with vehicle (saline) or test article (4.12 mg/kg of 3,4-DAPP) were animal specific and were initiated upon the first observance of the manifestation of select toxic signs associated with botulism (i.e. muscular weakness, respiratory distress, oral/nasal discharge). Repeat dosing was performed with a dosing interval of every 2 hours ± 15 minutes. Endpoints used to evaluate prolonged survival were survival (time to death), clinical observations, and clinical severity scores.

Defense Threat Reduction Agency was the Sponsor of the study. [redacted], was designated as the Sponsor Representative.

The study protocol and a summary of deviations are presented in Appendix A and Appendix B, respectively.

2 MATERIALS AND METHODS

2.1 CHALLENGE MATERIAL, TEST ARTICLE, AND VEHICLE

2.1.1 Challenge Material

The BoNT/A1 challenge material was identified as follows:

Product Name:	Botulinum neurotoxin complex serotype A1 (BoNT/A1)
Description:	BoNT/A1 stock solution provided by Metabiologics, Inc. Diluted in sterile 30 mM PBS containing 0.2% (w/v) gelatin (gelatin phosphate buffer; pH 6.2) to achieve a final dosing solution of 1,000 mouse intraperitoneal LD_{50}s/mL ($MIPLD_{50}$)
Batch/Lot Number:	A081508-01
Potency:	Stock potency solution determined by Battelle in May 2021 to be 521,330 $MIPLD_{50}$/mL.
Storage Conditions:	Frozen at ≤ -60°C until formulation, then stored at 2 to 8°C for up to 4 hours before administration.

Expiration Date:	Not assigned. Stability is assessed upon use per cumulative sum control charting (CUSUM) of assay performance and not on the original manufacturers' expiration date assignment of 08/15/2009.

The original manufacturers Certificate of Analysis (CoA) and updated potency characterization for the challenge material are included in Appendix C.

2.1.2 Test Article

The 3,4-diaminopyridine phosphate (3,4-DAPP) test article was identified as follows:

Product Name:	Amifampridine Phosphate Drug Substance (synonym 3,4-diaminoprydine phosphate; abbreviated 3,4-DAPP)
Batch/Lot Number:	P528FPM-19-002
Purity:	99.2% (by HPLC)
Storage Conditions:	Powder stored at controlled room temperature until reconstituted in saline at a final concentration of 2 mg/mL. Reconstituted test article was stored at 2 to 8°C for up to 6 hours prior to administration
Retest Date:	December 2022

A Certificate of Analysis and stability/retest information for the test article are included in Appendix D.

2.1.3 Vehicle

The vehicle used for preparation of the 3,4-DAPP dosing solutions and for the treatments of the vehicle control animals in Group 1 was identified as follows:

Name:	Sterile Saline or 0.9% Sodium Chloride for Injection USP
Manufacturer:	Covetrus/icumedical
Batch/Lot Number:	A2110132 (Covetrus); 24-701-FW(icumedical)
Expiration Date:	10/31/2024 (Lot A2110132) 12/1/2022 (Lot 24-701-FW)
Storage Conditions:	Controlled room temperature

Both of the lots listed were used to prepare the 3,4-DAPP dosing solutions. Lot A2110132 was used to prepare the 3,4-DAPP dosing solutions starting with the 1200 time point on April 12, 2022 through the 1200 time point on April 14, 2022. Lot 24-701-FW was used to prepare the 3,4-DAPP dosing solutions starting with the 1800 time point on April 14, 2022 through the final treatment time point at 2200 on April 16, 2022.

Lot 24-701-FW was the sole lot administered to the Group 1 animals.

A verified photocopy of the label characterization for each lot of saline used along with a Certificate of Analysis (CoA) for each is included in Appendix D.

2.2 CHALLENGE MATERIAL PREPARATION

One hundred (100) µL of BoNT/A1 stock (521,330 $MIPLD_{50}$/mL) was diluted into 9.9 mL of gel phosphate diluent to produce an initial diluted working solution (5,213.3 $MIPLD_{50}$/mL). A second dilution was performed by adding 1.919 mL of the working solution into 8.081 mL of gel phosphate diluent. The resulting formulation produced a total volume of 10 mL of a final BoNT/A1 dosing solution with a potency of 1,000 $MIPLD_{50}$/mL.

2.3 TEST ARTICLE PREPARATION

3,4-DAPP was prepared at a concentration of 2 mg/mL in 0.9% sodium chloride (sterile saline). 3,4-DAPP dose formulations were prepared fresh throughout the day and night when dosing activities were required. 3,4-DAPP solutions were maintained on wet ice after preparation and were replaced with fresh solutions within 6 hours.

Test Article Analysis Formulation analysis of the test article dosing solutions in saline was not performed.

2.4 RESERVE SAMPLES

Reserve samples were not collected nor required for this non-GLP study.

2.5 DISPOSITION OF CHALLENGE MATERIAL AND TEST ARTICLE

Unused frozen stocks of the challenge material were retained at the Battelle Biomedical Research Center (BBRC) for use on future BoNT studies. The residual challenge dosing solution was discarded according to facility procedures after the conclusion of the study.

Unused bulk test article was retained at the BBRC for use on future studies. Residual prepared test article dosing solutions were discarded after the conclusion of the study.

2.6 EXPERIMENTAL ANIMALS

A total of thirteen (13) male cynomolgus monkeys (of Mauritius origin) were transferred from the Battelle holding protocol (2995-C1176424) to Battelle Study B06118 on March 30, 2022.

Quarantine exams, fecal exams, tuberculous testing, physical exams, and clinical pathology health screens (hematology and serum chemistry) were performed prior to transfer to Battelle Study B06188.

Animals were weighed on April 8, 2022 (Study Day -3) for randomized group assignment by body weight. Male animals were approximately 3 to 4 years of age at group assignment with body weights ranging from 2.72 to 4.37 kg. To maximize the number of animals on study weighing greater than or as close to 3kg as possible, after randomization, prior to challenge, the animal with the lowest body weight (BL123C; 2.72kg) was replaced with the animal

originally randomized as an extra (BM162L; 4.37kg). The animal not used on study (BL123C), per Sponsor concurrence, was transferred back to the Battelle holding protocol (2995-C1176424) to be used for non-invasive training and/or possible use on future studies per Sponsor direction. A complete listing of animal identification numbers, study group assignments, birth dates, and body weights are presented below in Table 1.

Table 1. Group Assignment, Animal Identification, Age, and Body Weight

Animal ID	Date of Birth	Weight (kg)
Group 1 (Vehicle)		
AL403H	01/27/2019	4.21
SA179G	01/28/2019	2.97
BM842C	01/29/2019	3.27
BC114A	01/23/2019	3.67
JR81CB	08/22/2018	3.48
BM813G	01/28/2019	2.84
Group 2 (3,4-DAPP)		
AK950J	01/07/2019	3.57
SL42B	01/07/2019	3.46
BM162L	01/30/2019	4.37
AK394MB	01/29/2019	3.84
GA669H	01/28/2019	3.16
AR787L	01/30/2019	3.21

2.6.1 Housing and Environmental Conditions

General procedures for animal care and housing met current Association for Assessment and Accreditation of Laboratory Animal International (AAALAC) recommendations, current requirements stated in the "Guide for Care and Use of Laboratory Animals" (National Research Council), current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act, as amended, and conformed to testing facility SOPs.

Animals were housed in stainless steel cages and were socially housed (to the extent possible) during holding and prior to randomization. Following weighing and randomization on Day -3, animals were separated into single housing arrangements and remained single housed throughout the duration of the study. During periods of single housing, socialization was achieved by housing animals in the same room with visual and auditory contact.

The light/dark cycle was controlled by electronic timers set to maintain 12 hours of light and 12 hours of dark each day using fluorescent lighting set to start at 6:00 AM each day. Following challenge, the lighting cycle was suspended and lights were turned on for observation and study activities when required. Room temperature and relative humidity controls were set to maintain ranges of 64 to 84°F (set point of 74°F) and 30 to 70% (set point of 40.0%), respectively.

2.6.2 Feed and Water

Animals were offered PMI, Inc. Certified Monkey Chow. Analytical reports of each feed lot were provided by the manufacturer.

Fresh water from the Village of West Jefferson municipal water supply was provided *ad libitum* to the animals by an automatic watering system except when removed from the home cage.

2.6.3 Environmental Enrichment

Animals were provided fresh fruits or fresh vegetables and/or other enrichment items to promote and enhance psychological well-being. Additional environmental enrichment was provided in the room with a radio.

2.6.4 Animal Identification and Group Assignment

Animals were identified by the supplier's tattoo designation. Animals were weighed on April 8, 2022 (Study Day -3) and assigned to dose groups using a stratified randomization program (Provantis) designed to maintain similar group mean body weights.

3 EXPERIMENTAL DESIGN

Twelve (12) male cynomolgus monkeys were assigned to challenge and treatments groups as presented in Table 2 below.

Table 2. Study Design

Group	Animal ID	Challenge; Dose; Route	Treatment; Dose; Route*	Endpoints
1	AL403H	BoNT/A1; 2.5x LD_{50}; IM	Vehicle; 0 mg/kg; SC	Survival/Time to Death Clinical Observations Clinical Severity Scores
	SA179G			
	BM842C			
	BC114A			
	JR81CB			
	BM813G			
2	AK950J		3,4-DAPP; 4.12 mg/kg; SC	
	SL42B			
	BM162L			
	AK394MB			
	GA669H			
	AR787L			

IM = intramuscular; SC = subcutaneous
*Treatments initiated at the first sign of toxicity with a clinical severity score of 1 or greater. Repeat treatments delivered at a target interval of every 2 hours thereafter for up to 96 +/- 2-hours after first treatment.

3.1 CHALLENGE ADMINISTRATION

The challenge was conducted on a single study day. The day of challenge was defined as Study Day 1. Unanesthetized animals were chair restrained for the challenge administration procedures. Systemic intoxication with BoNT/A1 was achieved by a single IM injection of the BoNT/A1 dosing solution into the right calf muscle at a dose level of 97.5 $MIPLD_{50}$/kg, corresponding to 2.5x the LD_{50} for IM intoxication in cynomolgus monkeys with BoNT/A1 (Scott and Suzuki, 1988). Intramuscular challenge volumes ranged from 0.28 to 0.43 mL to achieve the specific challenge level based on animal weight. After challenge, animals were returned to their home caging for observation. A complete listing of BoNT/A1 challenge dose volumes is presented below in Table 3.

Table 3. Intramuscular BoNT/A1 Challenge Volumes

Group	Animal ID	BoNT/A1 Challenge Volume (mL)
1	AL403H	0.41
	SA179G	0.29
	BM842C	0.32
	BC114A	0.36
	JR81CB	0.34
	BM813G	0.28
2	AK950J	0.35
	SL42B	0.34
	BM162L	0.43
	AK394MB	0.37
	GA669H	0.31
	AR787L	0.31

3.2 TREATMENT ADMINISTRATION

Repeat treatment with 3,4-DAPP (or saline) was administered as a SC bolus at alternating sites on the hip/thigh and scapula area. After the conclusion of the first 11 treatment periods (April 13, 2022 @ 1200 through April 14, 2022 @ 1000), due to logistical and safety concerns associated with administering the dose into the hip/thigh area, the remainder of the treatments were restricted to the top and bottom region of the right and left scapula for the remainder of the study (Deviation Report (DR)-B06188-BBRC-0001, Appendix B). A clinical severity score of 1 or greater served as a trigger to initiate 3,4-DAPP or saline treatment. Repeat treatments were administered at a targeted 2-hour +/- 15-minute interval thereafter. Actual intervals between treatments ranged from 1 hour and 43 minutes to 2 hours and 35 minutes. A complete listing of treatment times and treatment intervals are provided in Appendix F.

Saline (0.9%) was administered as received without additional dilutions or preparations. Saline dosing volumes were matched to the dosing volume employed for 3,4-DAPP treatment groups. 3,4-DAPP was administered using a 2 mg/mL dosing solution, prepared by reconstituting lyophilized 3,4-DAPP in 0.9% saline solution. Working 3,4-DAPP solutions were maintained on ice after preparation for a maximum of 6 hours during administration after which any residual 3,4-DAPP solution was replaced with a fresh drug preparation. Animals receiving 4.12 mg/kg of 3,4-DAPP were dosed at 2.06 mL/kg. Dosing volumes for each group ranged from 5.9 to 8.7 mLs per treatment for Group 1 (saline) to 6.5 to 9.0 mLs per treatment for Group 2 (3,4-DAPP). A complete listing of treatment dose volumes and number of treatments is presented below in Table 4.

Table 4. Subcutaneous Treatments

Group	Treatment (dose)	Animal ID	Treatment Volume (mL)	Number of Treatments Administered	Total Volume Administered (mL)
1	Saline (0 mg/kg)	AL403H	8.7	16	139.2
		SA179G	6.1	18	109.8
		BM842C	6.7	19	127.3
		BC114A	7.6	13	98.8
		JR81CB	7.2	18	129.6
		BM813G	5.9	26	153.4
2	3,4-DAPP (4.12 mg/kg)	AK950J	7.4	27	199.8
		SL42B	7.1	25	177.5
		BM162L	9.0	37	333.0
		AK394MB	7.9	36	284.4
		GA669H	6.5	16	104.0
		AR787L	6.6	23	151.8

3.3 CLINICAL OBSERVATIONS AND SEVERITY SCORING

Observations for moribundity and mortality were performed a minimum of twice daily during the pre-study period.

On the day of challenge (Day 1), a morning observation was performed prior to BoNT/A1 administration. Following BoNT/A1 challenge, a single PM observation was performed. Scheduled routine observations were performed every 2 hours starting at 0800 the day after challenge (Day 2). Animals were observed for signs commonly associated with botulism (e.g., ptosis, muscular weakness, respiratory distress, oral/nasal discharge) and clinical severity scoring was performed according to Table 5 at each observation time-point (DR-B06188-BBRC-0004, Appendix B; two instances of omission/error of scoring value; no impact) .

Table 5. Clinical Severity Scoring

Clinical Signs	Observation	Clinical Severity Score Assigned
Ptosis	Absent	0
	Slight	1
	Marked	2
Muscular Weakness	Normal	0
	Hunched Posture	1
	Prostrate but able to rise	2
	Prostrate but unable to rise	3
Respiratory Distress	Normal breathing	0
	Open mouth shallow (thoracic)	1
	Gasping and jerking (abdominal)	3
Oral Discharge	Absent	0
	Present	1
Nasal Discharge	Absent	0
	Present	1
Lethality	Alive	0
	Dead	10

3.4 BODY WEIGHTS

Body weight checks were performed while the animals were being held under the Battelle animal holding protocol (2995-C1176424) on (in relation to Day 1 of this study) Day -328, Day -314, Day -300, Day -165, Day -146, Day -115, Day -73, and Day -41. A final body weight was collected on April 8, 2022 (Day -3). This weight was used for randomization, challenge dose calculations, and treatment dose calculations.

3.5 STUDY TERMINATION

The in-life phase of Battelle Study B06188 was concluded on April 16, 2022 (Day 6) when the final animal succumbed to challenge.

4 COMPUTER SYSTEMS FOR DATA MANAGEMENT

The following computer systems were used for toxicology data management:

Table 6. Computer Systems

Computer System	Version No.	Manufacturer	Data Type
ECMS	3.13	Siemens	Building Monitoring (Room Temperatures, Light Cycles, Pressure, Humidity, Air Flow)
Insight, InfoCenter Suite, and APOGEE	3.9.1		
Intemp Connect	Not available/applicable	Onset Computer Corporation	Cold Storage Unit Temperature monitoring
Provantis	10.1.0.0	Instem	Clinical observations, body weights, randomizations

5 STATISTICAL ANALYSIS

The endpoint for survival was defined by the number of animals in each group that were alive or dead at 96 hours (± 30 minutes) after the onset of a clinical severity score of 1 or greater in any one of the selected toxic signs. Survival proportions with exact 95% confidence intervals were calculated for each group. A log-rank test was used to compare time to death after toxic sign onset (clinical severity of ≥ 1) between the two groups. A log-rank test was also used to compare times to death from challenge between the two groups. A Kaplan-Meier plot was estimated relative to the time of toxic sign onset (clinical severity of ≥ 1).

The proportions of observed clinical signs with exact 95% confidence intervals were calculated for each group at each time point. At each time point where at least three animals survived in both groups, a two-sided Boschloo's test was used to compare the proportions of each clinical observation between the two groups. At each time point, the Bonferroni-Holm multiple comparison procedure was used to control the overall Type I error rate of the tests across the different clinical observations at 5% ($p \leq 0.05$). Across all time points, the numbers of animals that ever presented a clinical observation were calculated by group, and two-sided Boschloo's tests were used to compare between the two groups.

For total and respiratory severity scores, medians with interquartile ranges were calculated for each group at each time point. At each time point where at least three animals survived in both

groups, a Kruskal-Wallis test was performed to determine if there were significant differences between the group medians. Medians and interquartile ranges were plotted for the severity scores.

Contributing scientist reports containing the statistical analysis of the clinical observations, clinical severity scores and time to death are included in Appendix H and Appendix I.

6 STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

The final report and all records generated at the BBRC testing facility that would be required to reconstruct the study are archived at the Battelle archives.

Unused frozen bulk working stock aliquots of the BoNT/A1 used on this study are retained at the BBRC for use on future studies. The prepared BoNT/A1 challenge solution was discarded after use. The unused bulk stock of 3,4-DAPP powder was retained at the BBRC for use on future studies. Individual 3,4-DAPP dosing solutions and vehicle were discarded after use.

7 RESULTS

7.1 MORTALITY

Individual Mortality Data: Appendix G

Statistical Analysis of Time to Death: Appendix H

All 12 animals placed on study and challenged with BoNT/A1 succumbed to challenge prior to scheduled euthanasia and removal from the study at 96 ± 2 hours from the initiation of treatment. Mortality was expected following systemic exposure to BoNT/A1 at the challenge levels employed (2.5x LD_{50}) and was considered specifically attributed to administration of the challenge material.

Relative to the time of BoNT/A1 challenge (2.5x LD_{50}), time-to-death for Group 1 animals receiving saline treatment ranged from 85.23 hours to 111.20 hours. Post-challenge time-to-death for Group 2 animals receiving 4.12 mg/kg of 3,4-DAPP treatment ranged from 94.28 hours to 135.65hours. Table 7 contains the time-to-death after challenge and after the initiation of treatment for each animal in each group.

Given the varying post-challenge latency times it took for individual animals to reach trigger-to-treatment criteria, ranging from 53.23 hours to 67.45 hours, interpretation of mortality results strictly relative to challenge time does not account for survival time relative to treatment. As such, additional analysis was performed to investigate potential treatment effects on survival time after animals displayed toxicity and treatments were initiated. This was accomplished by examining the difference between time-to-death as it relates to the time-to-trigger criteria and may be interpreted as a more accurate measure of potential test article-effects on altering clinical trajectory after presentation of signs associated with botulism. For all animals, the first observation of toxicity corresponded to a clinical severity score of ≥ 1 which triggered the initiation of treatments.

Following post-challenge treatment trigger criteria (clinical severity score of ≥ 1), time-to-death for Group 1 animals receiving saline treatment ranged from 26.13 hours to 52.13 hours

with the median group time-to-death (accounting for all 6 animals in the group) of 35.38 ± 8.64 hours. Post-trigger time-to-death for Group 2 animals receiving 4.12 mg/kg of 3,4-DAPP treatment ranged from 30.75 hours to 73.90 hours with the median group time to death of 52.20 ± 16.43 hours. The log-rank test performed to compare times to death after treatment trigger showed a significant difference (p=0.0416) between Group 1 and Group 2. Time-to-death from the time of challenge ranged from 85.23 hours to 111.20 hours (median 94.38 ± 8.94 hours) for all animals receiving saline (Group 1) and from 94.30 hours to 135.65 hours (median 115.71 ± 13.95 hours) for all animals receiving 3,4-DAPP (Group 2). The log-rank test performed to compare times to death after challenge showed a significant difference between Group 1 and 2 (p=0.0049). Life extension attributed to animals receiving 3,4-DAPP (when compared to untreated controls) was 21.33 hours from the time of challenge and 16.82 hours from the time of treatment. A complete listing of challenge times, times to toxicity and trigger, and times to death relative to challenge, toxicity, and trigger are presented for all animals in Appendix G. An abbreviated summary is provided below in Table 7. Figure 1 presents a Kaplan-Meier plot relative to the time of onset by group.

Table 7. Time to Death by Group

Group	Median Time to Death After Challenge in hours(range)	STD (hours)	P-value	Median Time to Death After Treatment Trigger in hours (range)	STD (hours)	P-value
Saline Control	94.38 (85.23 - 111.20)	8.94	0.0049*	35.38 (26.13 – 52.13)	8.64	0.0416*
4.12 mg/kg 3,4-DAPP	115.71 (94.30 – 135.65)	13.95		52.20 (30.75 – 73.90)	16.43	
Median Post-Challenge Treatment Survival Benefit; 3,4-DAPP vs. saline control (hours)	21.33					
Median Post-Trigger Treatment Survival Benefit; 3,4-DAPP vs. saline control (hours)				16.82		

* Times to death were significantly different between groups STD = Standard Deviation

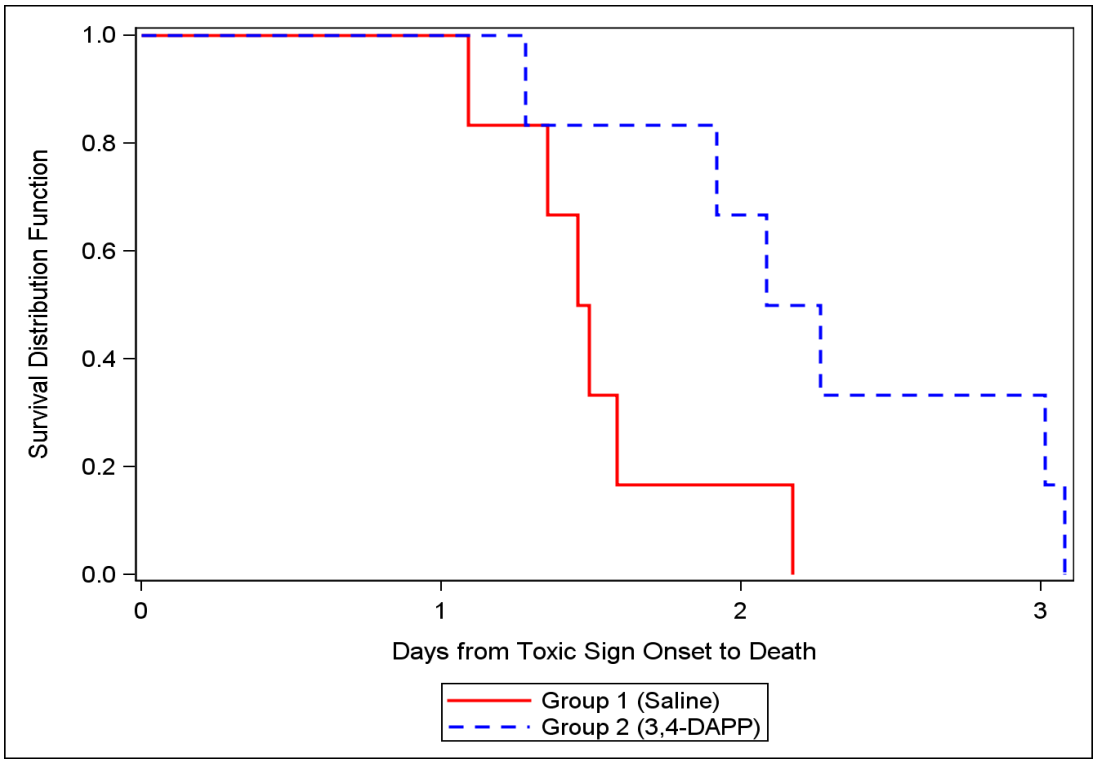


Figure 1. Kaplan-Meier Plot of Time to Death from Time to Onset of Toxin Signs by Group

Compared to saline-treated controls, repeat SC treatment with 3,4-DAPP at a dose-level of 4.12 mg/kg prolonged survival, as evidenced by a significantly greater median time-to-death value (~52 hours versus 35 hours; p = 0.0416) following initial presentation of signs associated with botulism. As such, indications of prolonged survival times following toxicity was considered attributable to test article administration at the 4.12 mg/kg dose level.

Appendix H contains the statistical analysis of the time to death comparison between Group 1 and Group 2.

7.2 CLINICAL OBSERVATIONS

Clinical Observations: Appendix G
Statistical Analysis of Clinical Observations: Appendix I

Regularly scheduled descriptive post-challenge observations were performed per the unit clock at 2 hour ± 15 minute intervals starting at 0800 on Study Day 2. With the exception of occasional non-botulinum stool-related issues (soft stool, diarrhea, etc.) commonly seen with this species, all animals were observed to remain in clinically normal status for approximately 53 hours post-challenge. The first observation of toxicity (ptosis) following challenge occurred at 53 hours and 14 minutes in Group 1 Animal JR81CB. All remaining animals continued to start showing initial signs of toxicity (ptosis, hunched posture and/or labored breathing) over the next 13 hours with the final animal remaining in clinically normal status until 67 hours and 27 minutes post-challenge (Group 2 Animal AR787L). The median initial time to toxicity was

very similar for both groups (~59 hours for Group 1 and ~63 hours for Group 2). Leading signs of intoxication (greater than 50 instances) included lethargy, hunched posture, labored breathing, ptsosis, ataxia, decreased respirations, and gasping. The number of abnormal observations increased over time as disease progressed. Other abnormal clinical signs observed following challenge were oral discharge, shallow respirations, nasal discharge, forced abdominal respirations, increased respirations, prostrate posture, and death. Table 8 contains a list of botulinum related observations per relevant time point (ranging from Day 3 at 2000 through Day 5 at 0600) where the proportion of animals observed displaying the sign was significantly greater (per Boschloo's tests) in the saline control group (Group 1) versus the group receiving 4.12 mg/kg 3,4-DAPP (Group 2).

All abnormal observations were considered specifically attributed to administration of the challenge agent (BoNT/A1) and similar to what was observed in Battelle Study B05929. In general, the overall severity and frequency of clinical observations following BoNT/A1 challenge was greater in the saline treated animals versus the animals administered 4.12 mg/kg of 3,4-DAPP. At the dosing frequency interval employed in this study (every 2 hours), SC treatment with 4.12 mg/kg of 3,4-DAPP did not completely prevent disease progression after initial presentation of toxicity as all animals on study succumbed to challenge prior to scheduled euthanasia at 96 ± 2-hours following initiation of treatment. Qualitative observations of acute toxicity reversal following 3,4-DAPP dosing was supported by a plateau or slight reduction in clinical severity scores at various times during the post-challenge observation and treatment period. Improvement effects were generally more frequent and more robust in animals receiving 3,4-DAPP (4.12 mg/kg) versus the animals receiving saline but became less effective at resolving toxicity at the late-stages of disease where toxic observations became numerous and more severe as mortality approached.

Appendix I contains additional statistical analysis of the group comparisons for all clinical observations by time point.

Table 8. Clinical Observation Time points with Significant Difference Between Control Versus Treated Animals

Day and Time	Clinical Observation						
	Hunched Posture	Lethargic	Decreased Respirations	Gasping	Ataxia	Oral Discharge	Prostrate
	X =Time points with significant differences (p-value)*						
Day 3 2000	X						
Day 3 2200	X						
Day 4 0000							
Day 4 0200	X						
Day 4 0400		X (0.0063)					
Day 4 0600		X (0.0063)					
Day 4 0800		X (0.0063)					
Day 4 1000		X (0.0063)					
Day 4 1200	X	X (0.0005)	X (0.0063)				
Day 4 1400		X (0.0005)					
Day 4 1600		X (0.0063)		X			
Day 4 1800		X (0.0063)		X			
Day 4 2000		X (0.0386)		X			
Day 4 2200		X (0.0386)			X	X (0.0210)	
Day 5 0000							
Day 5 0200	X						
Day 5 0400	X						X
Day 5 0600	X						X

* All p-values indicate proportion of animals displaying sign in saline control group (Group 1) was significantly greater than the group treated with 3.4-DAPP(Group 2).

7.3 CLINICAL SEVERITY SCORING

Clinical Severity Scores: Appendix G
Clinical Analysis of Severity Scores: Appendix I

All animals were observed with clinical severity scores of 0 on Day 1 prior to BoNT/A1 challenge. Following challenge, all animals remained in clinically normal status until the afternoon of Day 3 where two animals, JR81CB from Group 1 and AK394MB from Group 2 displayed a clinical severity score of 1. The time-to-first clinical severity score of ≥ 1 following challenge for all animals ranged from 53 hours and 14 minutes to 67 hours and 27 minutes. Clinical severity scores initially presented at a range of 1 to 2 in all animals treated with 3,4-DAPP and at a range of 1 to 3 in all animals treated with saline. As toxicity progressed from initial presentation of 1 or 2, clinical severity scores in most animals administered 3,4-DAPP generally continued to rise and fall between 1 to 5 prior to mortality. Animals administered saline generally showed slightly more severe scores as toxicity progressed, ranging from 2 to 8. Presentation of clinical severity scores of 10 (death) was expected following systemic exposure to BoNT/A1 at the challenge level employed (2.5x LD_{50}) and was considered specifically attributed to administration of the challenge material.

In general, the overall frequency and severity of clinical observations following BoNT/A1 challenge was higher in the group receiving saline versus the group receiving 4.12 mg/kg 3,4-DAPP. Three out of six animals (AR787L, AK950KJ and BM162L) receiving 3,4-DAPP had lower or equivalent severity scores 24-hours (12 treatments received) after receipt of the first treatment when compared to the scores recorded 8 hours (4 total treatments received) following the first treatment. The three additional animals in the group (GA669H, SL42B, AK934MG) showed just a single or two-point increase in severity during the same time frame. One (1) animal (BM162L) did show a return to a severity score of "0" between 12 to 18 hours following initiation of treatment before increasing from "1" to "5" prior to death. Interestingly, the animal (BM162L) in the group receiving 3,4-DAPP with the highest recorded severity score at any time point (5) was also the longest survivor in the group, surviving at least 8 hours longer than any other animal in the group.

By comparison, the median total severity scores of the saline control group were significantly greater ($p = 0.0056 – 0.0484$) than those of the 3,4-DAPP treated group at most time points starting from 2000 of Day to 0600 of Day 5. In general, as disease progressed, clinical severity scores in the animals receiving saline were at least 1 to 4 points higher than the animals receiving 3,4-DAPP at the same time points. Similar to the 3,4-DAPP group, the animal with the highest recorded severity score at any time point (8) was the longest survivor in the group, surviving greater than 12 hours longer than any other animal in the group.

A summary table of each clinical severity score for each animal at each time point is presented in Table 9. A summary table of median clinical severity scores (with interquartile range) by group through the first 24 time points (the last timepoint where survival data from greater than 50% of the animals from each group are represented) is presented in Table 10. A plot of the median total severity scores for each group at each time point showing interquartile ranges and noting timepoints of significant differences is presented in Figure 2,. Severity scores of 10 (death) were excluded. As presented in the figure, starting six hours after all animals had started treatments (Day 4, 1000), the median clinical severity scores at each proceeding time point (through Day 5, 0600) for the group receiving 3,4-DAPP were significantly lower ($p = 0.0073 – 0.0484$) than the mean clinical severity scores for the group receiving saline, ranging in a group mean difference from 1.0 to 4.0.

Table 9. Clinical Severity Scores by Individual Animal and Time Point

	Treatment											
	3,4-DAPP						SALINE					
	Animal ID						Animal ID					
Tot	GA669H	AR787L	SL42B	AK950J	AK394MB	BM162L	BM813G	SA179G	BM842C	JR81CB	BC114A	AL403H
	Clinical Severity Score											
Day 1	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1200	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1400	0	0	0	0	1	0	0	0	0	1	0	0
Day 3 1600	0	0	0	0	1	0	0	0	0	1	0	0
Day 3 1800	0	0	0	0	1	0	0	0	0	1	0	0
Day 3 2000	0	0	0	0	1	0	2	1	1	2	1	0
Day 3 2200	0	0	0	0	1	1	2	2	1	3	1	1
Day 4 0000	1	0	1	2	1	1	2	2	2	3	1	2
Day 4 0200	1	0	1	2	1	1	2	2	2	3	1	2
Day 4 0400	1	2	2	2	4	1	4	3	3	4	2	2
Day 4 0600	1	2	2	2	4	2	4	3	3	3	3	2
Day 4 0800	2	2	2	1	4	1	2	3	3	3	4	3
Day 4 1000	2	2	2	1	4	0	2	4	3	3	5	3
Day 4 1200	2	2	2	1	3	0	3	5	3	3	5	3
Day 4 1400	3	2	2	1	3	0	3	5	3	3	5	3
Day 4 1600	3	2	2	1	3	0	3	5	3	3	3	3
Day 4 1800	3	2	2	1	3	1	3	5	5	3	4	3
Day 4 2000	3	2	2	1	3	1	3	5	5	3	5	5
Day 4 2200	3	3	2	1	3	1	5	5	4	3		5
Day 5 0000	2	1	2	1	2	1	2	4	3	3		4
Day 5 0200	3	1	2	1	2	1	4	4	3			5
Day 5 0400	3	1	2	1	2	1	5	4	4			5
Day 5 0600	3	1	1	1	1	1	5	4	5			5

Table 9. Clinical Severity Scores by Individual Animal and Time Point (Continued)

	Treatment											
	3,4-DAPP						SALINE					
	Animal ID						Animal ID					
Tot	GA669H	AR787L	SL42B	AK950J	AK394MB	BM162L	BM813G	SA179G	BM842C	JR81CB	BC114A	AL403H
	Clinical Severity Score											
Day 5 0800		1	2	1	1	1	5		5			
Day 5 1000		2	2	1	1	2	5					
Day 5 1200		2	2	1	1	2	5					
Day 5 1400		2	2	1	1	1	7					
Day 5 1600		2	2	1	1	1	7					
Day 5 1800		2	2	1	1	1	7					
Day 5 2000		2	3	1	1	3	7					
Day 5 2200		2	2	1	1	5	8					
Day 6 0000		4	3	4	3	5						
Day 6 0200				4	3	4						
Day 6 0400				4	3	4						
Day 6 0600				5	3	4						
Day 6 0800					3	4						
Day 6 1000					3	5						
Day 6 1200					3	5						
Day 6 1400					5	5						
Day 6 1600						5						
Day 6 1800						5						
Day 6 2000						5						
Day 6 2200						5						

Table 10. Descriptive Statistics for Total Severity Scores by Group and Time Point (through last time point with at least 50% survival in both groups)

Time Point	Group 1 – Saline	Group 2 – 3,4-DAPP	P-value
Day 1	0.0	0.0	1.0000
Day 2	0.0	0.0	1.0000
Day 3 1200	0.0	0.0	1.0000
Day 3 1400	0.0	0.0	1.0000
Day 3 1600	0.0	0.0	1.0000
Day 3 1800	0.0	0.0	1.0000
Day 3 2000	1.0	0.0	0.0231*
Day 3 2200	1.5	0.0	0.0110*
Day 4 0000	2.0	1.0	0.0248*
Day 4 0200	2.0	1.0	0.0248*
Day 4 0400	3.0	2.0	0.0931
Day 4 0600	3.0	2.0	0.0750
Day 4 0800	3.0	2.0	0.0668
Day 4 1000	3.0	2.0	0.0484*
Day 4 1200	3.0	2.0	0.0073*
Day 4 1400	3.0	2.0	0.0163*
Day 4 1600	3.0	2.0	0.0200*
Day 4 1800	3.5	2.0	0.0123*
Day 4 2000	5.0	2.0	0.0077*
Day 4 2200	5.0	2.5	0.0107*
Day 5 0000	3.0	1.5	0.0105*
Day 5 0200	4.0	1.5	0.0122*
Day 5 0400	4.5	1.5	0.0089*
Day 5 0600	5.0	1.0	0.0056*

Data beyond the Day 5 at 0600 is not included in this table as Group 1 (saline) contained less than 50% survivors beyond this time point. Severity scores of 10 (death) were excluded.
*Median severity scores were significantly different between the groups (per Kruskal-Wallis test)

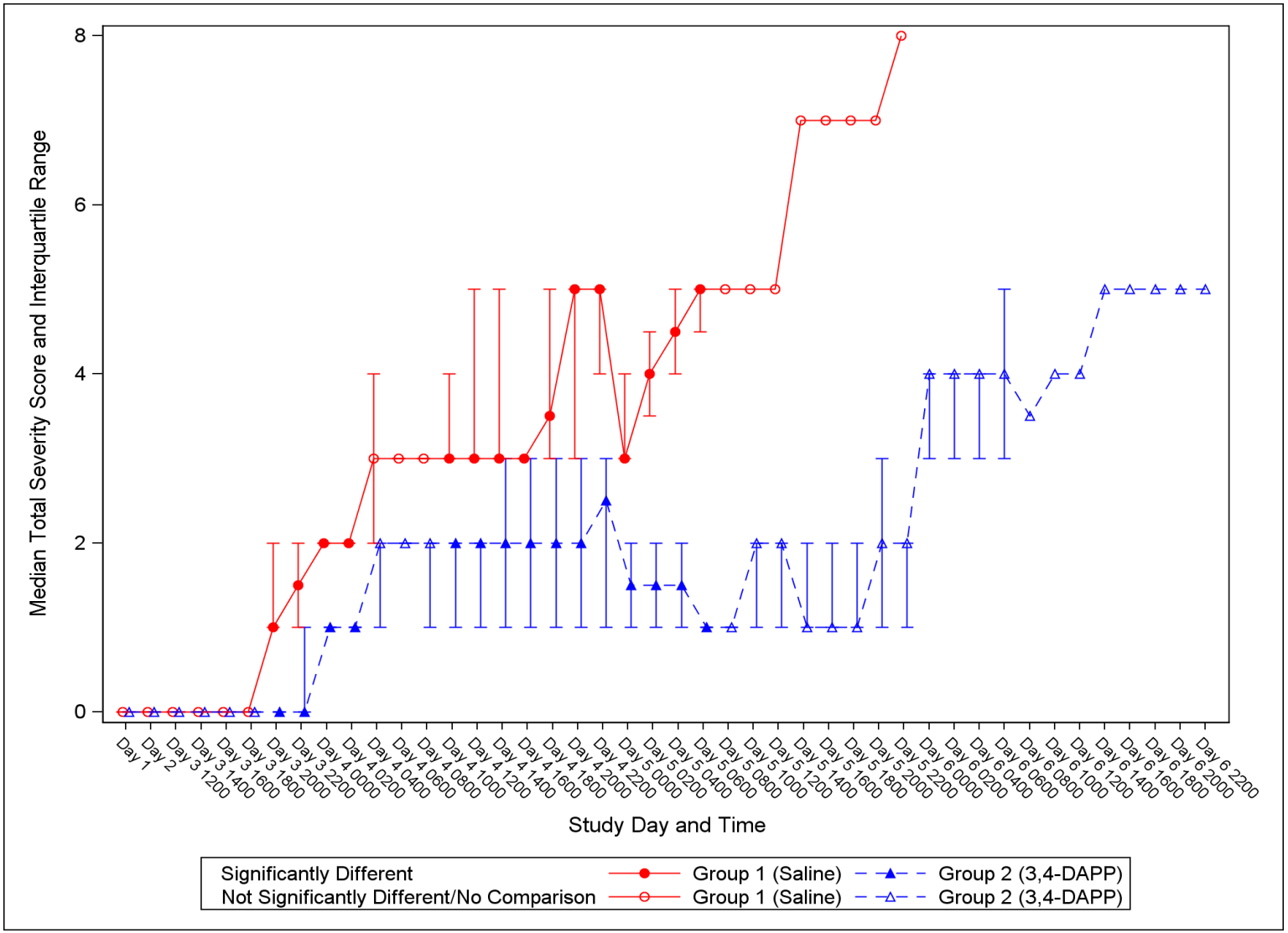


Figure 2. Median Total Severity Scores per Time Point and Group (with interquartile range and time points notated for differences of significance)

Table 11 and Figure 2 presents the clinical severity scores (in numerical and graph form) associated with only respiratory signs for each individual animal at each available time point while Table 12 and Figure 3 presents the mean clinical severity scores by group associated with only respiratory signs in the same manner during the same time frame. Signs recorded with severity scores of "1" or greater included labored breathing, shallow respirations, decreased respirations, increased respirations, gasping (all associated with a severity score of "`1") and forced abdominal respirations (associated with a severity score of "3"). Similar to the scores for total severity, although not as substantial of a difference, starting in the AM of Day 4 as toxicity progressed, scores associated with respiratory distress for the animals receiving saline were, in general, more prevalent across the entire group versus the animals receiving 3,4-DAPP and in two animals became more severe for at least two time points prior to death. Four of the six the animals receiving 3,4-DAPP, once reaching a severity score of "1" saw scores return to "0" for at least 10 to 24 hours before returning to a score of "1" and did not progress beyond that severity score prior to death. Similarly, the other two animals in the group, once reaching a score of "1" or "3" either stayed at or returned to a score of "1" for the remaining 10 or 22 hours prior to death.

Appendix I contains additional statistical analysis of the clinical severity scores by group and time point.

Table 11. Clinical Respiratory Severity Scores by Individual Animal and Time Point

	Treatment											
	3,4-DAPP						SALINE					
	Animal ID						Animal ID					
Time Point	GA669H	AR787L	SL42B	AK950J	AK394MB	BM162L	BM813G	SA179G	BM842C	JR81CB	BC114A	AL403H
	Respiratory Severity Score											
Day 1	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1200	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1400	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1600	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 1800	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 2000	0	0	0	0	0	0	0	0	0	0	0	0
Day 3 2200	0	0	0	0	0	0	0	0	0	1	0	0
Day 4 0000	1	0	0	1	0	0	0	0	0	1	0	1
Day 4 0200	1	0	0	1	0	0	0	0	0	1	0	1
Day 4 0400	1	1	1	1	1	0	1	0	1	1	1	1
Day 4 0600	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 0800	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 1000	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 1200	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 1400	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 1600	1	1	1	1	1	0	1	1	1	1	1	1
Day 4 1800	1	1	1	1	1	0	1	1	3	1	1	1
Day 4 2000	1	1	1	1	1	0	1	1	3	1	1	1
Day 4 2200	1	1	1	1	1	0	1	1	1	1		1
Day 5 0000	1	0	0	0	0	0	0	1	1	1		0
Day 5 0200	1	0	0	0	0	0	1	1	1			1
Day 5 0400	1	0	0	0	0	0	1	1	1			1
Day 5 0600	1	0	0	0	0	0	1	1	1			1
Day 5 0800		0	0	0	0	0	1		1			
Day 5 1000		1	1	0	0	0	1					
Day 5 1200		1	1	0	0	0	1					
Day 5 1400		1	1	0	0	0	3					
Day 5 1600		1	1	0	0	0	3					

Table 11. Clinical Respiratory Severity Scores by Individual Animal and Time Point (Continued)

	Treatment											
	3,4-DAPP						SALINE					
	Animal ID						Animal ID					
Time Point	GA669H	AR787L	SL42B	AK950J	AK394MB	BM162L	BM813G	SA179G	BM842C	JR81CB	BC114A	AL403H
	Respiratory Severity Score											
Day 5 1800		1	1	0	0	0	3					
Day 5 2000		1	1	0	0	0	3					
Day 5 2200		1	1	0	0	3	3					
Day 6 0000		1	1	1	1	1						
Day 6 0200				1	1	1						
Day 6 0400				1	1	1						
Day 6 0600				1	1	1						
Day 6 0800					1	1						
Day 6 1000					1	1						
Day 6 1200					1	1						
Day 6 1400					1	1						
Day 6 1600						1						
Day 6 1800						1						
Day 6 2000						1						
Day 6 2200						1						

Table 12. Descriptive Statistics for Clinical Severity Scores Associated with Respiratory Signs by Group and Time Point (through last time point with at least 50% survival in both groups)

Time Point	Group 1 – Saline	Group 2 – 3,4-DAPP	P-Value
Day 1	0.0	0.0	1.0000
Day 2	0.0	0.0	1.0000
Day 3 1200	0.0	0.0	1.0000
Day 3 1400	0.0	0.0	1.0000
Day 3 1600	0.0	0.0	1.0000
Day 3 1800	0.0	0.0	1.0000
Day 3 2000	0.0	0.0	1.0000
Day 3 2200	0.0	0.0	0.3173
Day 4 0000	0.0	0.0	1.0000
Day 4 0200	0.0	0.0	1.0000
Day 4 0400	1.0	1.0	1.0000
Day 4 0600	1.0	1.0	0.3173
Day 4 0800	1.0	1.0	0.3173
Day 4 1000	1.0	1.0	0.3173
Day 4 1200	1.0	1.0	0.3173
Day 4 1400	1.0	1.0	0.3173
Day 4 1600	1.0	1.0	0.3173
Day 4 1800	1.0	1.0	0.1757
Day 4 2000	1.0	1.0	0.1757
Day 4 2200	1.0	1.0	0.3613
Day 5 0000	1.0	0.0	0.1561
Day 5 0200	1.0	0.0	0.0143*
Day 5 0400	1.0	0.0	0.0143*
Day 5 0600	1.0	0.0	0.0143*

* Medians were significantly different between the groups (per Kruskal-Wallis test)

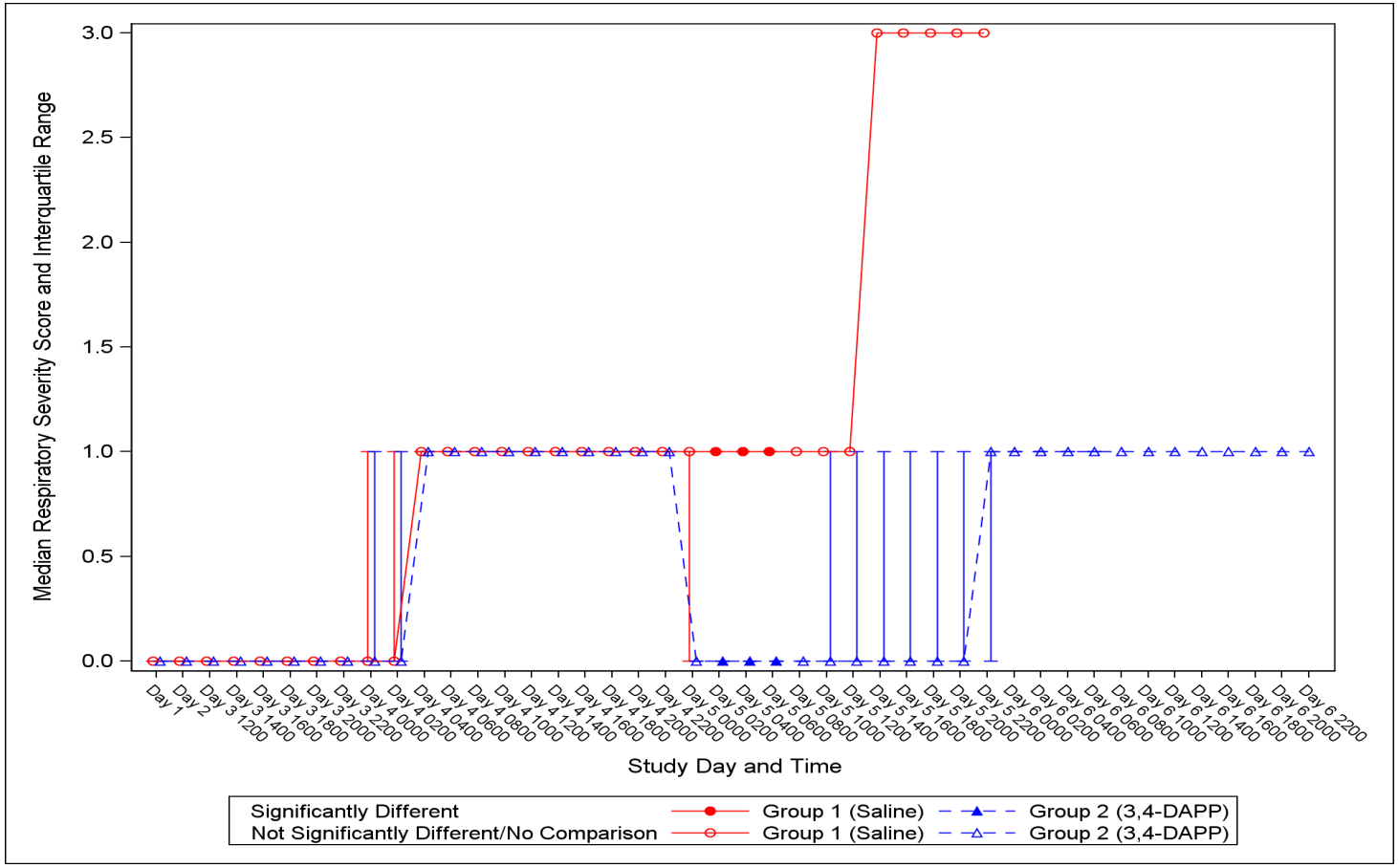


Figure 3. Median Clinical Respiratory Severity Scores per Time Point and Group (with interquartile range and time points notated for differences of significance)

8 DISCUSSION

The primary objective of this study was to evaluate the potential of 3,4-DAPP to prolong survival in cynomolgus monkeys systemically intoxicated BoNT/A1 and potential reversal of BoNT/A1 symptoms (i.e. paralysis).

In this study, a previously developed cynomolgus monkey (*Macaca fascicularis)* BoNT/A1 challenge model was used to complete 3,4-DAPP efficacy testing (Beske, 2019) using a lethal IM challenge dose of 2.5xLD_{50} of BoNT/A1 (97.5 $MIPLD_{50}$/kg; Scott and Suzuki, 1988).

Results obtained in this study were consistent with previous published data as well as a previous Battelle study examining the therapeutic efficacy of 3,4-DAPP (Battelle Study B05929), which observed induction of systemic toxicity within ~2.2 to 2.8 days after BoNT/A1 challenge. Similarly, after the manifestation of early toxic signs consistent with the onset of systemic botulism (i.e. muscle weakness, oral/nasal discharge), disease progression led to severe respiratory distress and eventual death.

The therapeutic benefit of post-symptomatic 3,4-DAPP treatment given every 2-hours was supported by evidence of sustained survival following the onset of systemic botulism. In Battelle Study B05929, BoNT/A1 challenged cynomolgus monkeys receiving 4.12 mg/kg of 3,4-DAPP every 4 hours, survived an average of 29 hours (19.90 hours to 38.07 hours) from the start of treatments and approximately 10 hours longer than the animals administered saline. In comparison, animals administered 4.12 mg/kg of 3,4-DAPP every 2 hours in the current study survived an average of 54 hours and 34 minutes (30.75 hours to 73.90 hours) from the start of treatments and approximately 17 hours longer than the animals receiving saline. All animals receiving 4.12 mg/kg of 3,4-DAPP every 2-hours survived beyond 30 hours after the onset of signs associated with botulism.

The current study compared to saline-treated controls with cynomolgus moneys receiving repeat SC treatments of 3,4-DAPP at a dose-level of 4.12 mg/kg (Section 7.1). Results show prolonged survival, as demonstrated by significantly greater median time-to-death values (~52 hours of the treated group versus 35 hours of the control group; p=0.0416) following initial presentation of signs associated with BoNT/A1.

The results obtained in this study offer promising preliminary data that could be used to support use of "Animal Rule" for approval of 3,4-DAPP under the using survival as a primary endpoint of efficacy (U.S. Food and Drug Administration, 2015). Consistent with Battelle Study B05929, utilizing at least a 4.12 mg/kg dose of 3,4-DAPP, shortening the dosing interval or having a continual level maintained in the bloodstream may confer additional prolongation of survival as results obtained in this study suggest that the durability in maintaining the reduced clinical severity effect was not always sustained from SC administration every two hours. 3,4-DAPP is the only reported botulism reversal agent (Siegel *et al.* 1986, Mayorov *et al.* 2010; Friggeri *et al.* 2013; Beske *et al.* 2017; Bradford *et al.* 2018; Beske, 2019; Vazquez-Cintron *et al*. 2020). The data collected in this study are generally consistent with these findings as well as findings from the previous Battelle Study B05929. Evidence of toxicity reversal or slowing of the progression of toxicity was apparent through qualitative clinical severity scoring and/or prolonged time to death. Early administrations of 3,4-DAPP at 4.12 mg/kg during the initial stages of BoNT/A1 exposure improved overall animal health as indicated by lower clinical

severity scores. As demonstrated, starting at six hours after all animals had started treatments (Day 4, 1000), the median clinical severity scores at each proceeding time point (through Day 5, 0600) for the group receiving 3,4-DAPP were significantly lower ($p = 0.0073 – 0.0484$) than the mean clinical severity scores for the group receiving saline, ranging in a group mean difference from 1.0 to 4.0 (Section 7.3, Figure 3).

Clinical severity scores were reduced following 4.12 mg/kg of 3,4-DAPP treatment and remained lower compared to the saline group. The finding from this study that 3,4-DAPP can improve neuromuscular function after the onset of botulism is consistent with pilot studies performed in humans (Friggeri *et al*. 2013). Similarly, consistent to pilot human studies, the magnitude of improvement appeared to vary among subjects, with some animals showing a greater improvement following treatment than others. The variability observed in this study likely mimics scenarios that would expected to be encountered in the real world.

The data from this study showing an average life extension of approximately 17 ± 8 hours after treatment with 3,4-DAPP supports the capacity of 3,4-DAPP to prolong survival following exposure to BoNT/A1.

9 ACKNOWLEDGEMENTS

Acknowledgements of the principal contributors participating in the performance of this study are presented below.

Table 13. Principal Contributors

Role	Name
Study Director	[redacted]
Lead Animal Technicians	[redacted]
Attending Veterinarian	[redacted]
Lead Chemist	[redacted]
Statistician	[redacted]
Quality Control Review	[redacted]

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Battelle Study No. B05929. *Efficacy Evaluation of 3,4-Diaminopyridine to Acutely Reverse Toxicity in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1.*

Appendix A Protocol

Survival Efficacy Evaluation of 3,4-Diaminopyridine in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1

Final Study Protocol

March 22, 2022

Study Number B06188

Defense Threat Reduction Agency
Chemical & Biological Technologies Department
Vaccines/Therapeutics Division (CBM)
8725 John J Kingman Rd MS 6201, Fort Belvoir, VA 22060

GLP Compliance Status: non-GLP

Battelle Memorial Institute
505 King Ave
Columbus, OH, 43201

Testing Facility:
Battelle Biomedical Research Center
1425 Plain City-Georgesville Road
West Jefferson, OH 43162

SIGNATURES

APPROVED, TESTING FACILITY:

[redacted] 29 Mar 2022
Study Director Date

[redacted] 3/29/2022
Date

[redacted]
Sponsor Representative Date
Defense Threat Reduction Agency

CDC Select Agent PI Assurance

The CDC Principal Investigator has read and reviewed the following protocol. All methods pertaining to the production, use, storage, and destruction of Biological Select Agent or Toxin are in accordance with Battelle approved procedures that adhere to the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.

CDC Principal Investigator: [redacted]

Signature/Date

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1.0 INTRODUCTION

Botulinum neurotoxins (BoNTs) are bacterial protein toxins that include antigenically distinct serotypes, labeled A-G [1]. Debate continues as to the presence of other unique serotypes, including BoNT/H [2] and BoNT/X [3]. The toxin itself consists of a heavy chain, which mediates neuron-specific binding and uptake, and a catalytically active light chain. Upon translocation to the presynaptic cytosol, the light chain proteolytically cleaves one of three SNARE proteins critically involved in neurotransmitter exocytosis: synaptosomal-associated protein 25 (BoNT/A, BoNT/C, BoNT/E); synaptobrevin-1/2 (BoNT/B, BoNT/D, BoNT/F, BoNT/G); or syntaxin (BoNT/C) [4]. The resulting inactivation of SNARE-dependent neurotransmitter release prevents the activation of postsynaptic receptors, ultimately manifesting in a flaccid paralysis. Depending on the serotype, intoxication can persist from weeks to months. At lethal doses, paralysis of muscles involved in respiratory function can lead to death by asphyxiation.

While the post-exposure administration of antibody-based antitoxin can extract and inactivate circulating BoNT, any toxin that has already entered the presynaptic compartment is inaccessible to antibody treatments. As such, the current post-exposure BoNT countermeasure approach is rendered largely ineffective after clinical manifestation of disease. Further complicating matters, the criteria for administration of antitoxin requires clinical evidence of flaccid paralysis. As such, even patients who receive antitoxin in a timely manner are likely to experience some symptoms of botulism. In severe cases of poisoning, the only treatment options include intensive supportive care (e.g., mechanical ventilation and parenteral nutrition), which may be needed for weeks to months once flaccid paralysis has manifested. Thus, there exists a critical need for novel late-acting post-symptomatic BoNT countermeasures that acutely reverse botulism symptoms, delay lethality, and/or accelerate disease recovery.

A promising candidate countermeasure to therapeutically treat the symptoms associated with botulism is 3,4-diaminopyridine (3,4-DAP). 3,4-DAP exerts positive modulation of neurotransmitter release through K^+ channel antagonism [5], thereby prolonging action potential duration, increasing presynaptic Ca^{2+} uptake, and potentiating Ca^{2+}-dependent vesicle acetylcholine release. Given the recent approval (November 2018) from the US Food and Drug Administration for Catalyst Pharmaceutical, Inc. to market 3,4-DAP phosphate (3,4-DAPP) (brand name Firdapse®) as a symptomatic treatment for Lambert-Eaton myasthenic syndrome (LEMS), an accelerated pathway towards repurposing 3,4-DAP as a BoNT countermeasure may now be possible. As non-clinical studies performed in NHPs are seen as an important part of predicting the human efficacy of candidate countermeasures, demonstrating initial proof-of-concept therapeutic benefit of 3,4-DAP in BoNT-intoxicated NHPs is an important step prior to further efficacy evaluation.

2.0 PURPOSE

Following systemic intoxication with botulinum neurotoxin serotype A1 (BoNT/A1), the objective of this study is to evaluate the capacity of 3,4-diaminopyridine (3,4-DAP) to promote survival in the cynomolgus monkey model.

3.0 REGULATORY COMPLIANCE

This study will be conducted consistent with, but not in strict compliance with, the current version of the United States Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58 for the conduct of non-clinical laboratory studies.

Any deviations from the study protocol will be documented in the study file and presented in the final report. Deviations from GLP and SOPs will be documented in the study file and presented in the final report as warranted.

4.0 TESTING FACILITY AND STUDY DIRECTOR

4.1 TESTING FACILITY

Battelle Biomedical Research Center
1425 Plain City-Georgesville Road
West Jefferson, OH 43162

4.2 STUDY DIRECTOR

[redacted]

5.0 SPONSOR AND SPONSOR'S REPRESENTATIVE

5.1 SPONSOR

Defense Threat Reduction Agency
Chemical & Biological Technologies Department
Vaccines/Therapeutics Division (CBM)
8725 John J Kingman Rd MS 6201, Fort Belvoir, VA 22060

5.2 SPONSOR'S REPRESENTATIVE

[redacted]

6.0 PROPOSED STUDY SCHEDULE

Proposed dates for selected study events are listed below.

In-Life:	February 2022
Sample Analysis and Reporting:	February 2022 – June 2022

7.0 CHALLENGE MATERIAL, TEST ARTICLE, AND PLACEBO

Paper documentation and/or the Inventory and Dispense modules under Provantis will be utilized for challenge material, test article or placebo inventory and preparation. Records of receipt, use, and distribution of materials used on study will be maintained in the study file.

7.1 CHALLENGE MATERIAL

Name:	Botulinum neurotoxin serotype A1 (BoNT/A1).
Description:	BoNT/A1 provided by Metabiologics, Inc. Diluted in sterile 30 mM PBS (pH 6.1-6.3) containing 0.2% (w/v) gelatin to achieve a final dosing solution of 1,000x mouse intraperitoneal LD_{50s}/mL ($MIPLD_{50}$)
Batch/Lot Number:	A081508-01
Storage Conditions:	Frozen at ≤ -60°C until formulation, then 2-8°C for up to 4 hours before administration.
Stability:	Assess upon use per cumulative sum control charting (CUSUM) of assay performance.
Expiration Date:	None assigned

The BoNT/A1 used on study is currently stored at Battelle. The provided certificate of analysis (CoA) for the specific lot used on study will be maintained in the study file. Documentation of synthesis will be maintained by the manufacturer.

The final potency value ($MIPLD_{50}$/mL) of the most recent mouse potency assay with the specific lot of challenge material to be used on study will be used to determine appropriate dilutions for preparation of final BoNT/A1 dosing solutions with a target potency of 1,000x $MIPLD_{50}$/mL.

7.2 TEST ARTICLE

Name:	3,4-diaminopyridine phosphate (synonyms: Amifampridine Phosphate Drug Substance; 3,4-DAPP)
Manufacturer:	Patheon (Greenville, SC, USA)
Description:	Active Pharmaceutical Ingredient (API) powder of Firdapse®; provided by Catalyst Pharmaceuticals. Final dosing solutions in saline will be prepared by Battelle. All dosing solutions will be prepared fresh on the day of use, maintained at 2-8°C when not in use, and will be visually inspected prior to administration.
Batch/Lot Number:	Lot number to be included in final report
Storage Conditions:	Controlled room temperature (CRT) until formulation, then a target of 2-8°C for up to 6 hours before administration
Stability:	Stable in powder form at CRT until re-test date.
Expiration Date:	To be included in final report

3,4-DAPP will be provided to Battelle by Catalyst Pharmaceuticals. A Certificate of Analysis (CoA) and/or equivalent documentation of test article identity, strength, purity, composition and other defining characteristics will be obtained for a specific lot utilized. Documentation of synthesis will be maintained by the manufacturer.

The concentration of dosing solutions (2 mg/mL) will be prepared so as to allow subcutaneous (SC) dose administration to remain within IACUC-approved SC dose administration volumes (5 mL/kg).

7.3 VEHICLE

Name:	0.9% sodium chloride for injection

Batch/Lot Number:	To be included in final report
Storage Conditions:	Controlled room temperature
Stability:	Stable at room temperature until the expiration date when stored according to the manufacturer instructions.
Expiration Date:	To be included in final report

The saline (0.9% sodium chloride for injection) for dose solution preparation will be characterized by its labeling. Documentation of synthesis will be maintained by the manufacturer as applicable. A copy of the package insert or Certificate of Analysis (CoA) and/or equivalent documentation of control article identity, strength, purity, composition and other defining characteristics will be obtained for a specific lot utilized, if possible.

8.0 TEST SYSTEM

Species:	Cynomolgus Monkey, *Macaca fascicularis*
Strain:	Mauritius Origin
Source:	PreLabs (Lehigh Acres, FL)
Anticipated Age of Animals on Day 1:	2 to 4 years, but not a requirement for placement on study
Anticipated Weight of Animals on Day 1:	3.0-5.0 kilograms
Number of Animals Required for Study:	13 total males (12 on study, 1 extra)
Special Considerations:	None

8.1 ANIMALS

Animals will be research naïve and will not have been utilized as part of the test system on previous studies. Only animals in good health, free of malformations that would otherwise interfere with the study and exhibiting no signs of clinical disease will be used on study. Animals that do not meet these criteria will be replaced by animals assigned as extras (if available). Extra animals(s) may be used as replacements on study as necessary including in events of unexpected mortality or technical difficulty.

8.2 ANIMAL IDENTIFICATION

Tattoos and cage cards will be used to identify each animal. Tattoo placement will have occurred prior to this study. The cage card will state, at a minimum, the species/strain, tattoo number, study number, and gender.

9.0 EXPERIMENTAL DESIGN

This study will be conducted in accordance with the protocol, protocol amendments, and applicable Battelle SOPs. An abbreviated list of procedures used in this study can be found in Section 17.0. This study will evaluate the proof-of-concept efficacy of 3,4-DAP to promote survival in BoNT/A1-intoxicated cynomolgus monkeys.

Prior to challenge (Day -3, Day -2, or Day -1), animals will be weighed for dose calculations and the challenge site will be clipped. On the day of challenge (Study Day 1), animals will be chair restrained and a single intramuscular (IM) injection of BoNT/A1 will be injected into the right calf muscle. The IM volume for challenge administration will not exceed 0.5 mL. BoNT/A1 will be delivered at a target dose of 97.5 MIP_{LD50s}/kg, corresponding to 2.5x the published IM LD_{50} of BoNT/A for cynomolgus monkeys [6].

Following BoNT/A1 administration, twice daily AM/PM observations will be performed for the first 24 hours after challenge as manifestation of toxic signs is not anticipated during this timeframe. From ~24 hours after challenge until study termination at mortality or scheduled euthanasia, animals will be clinically monitored at 2-hour intervals (± 15 minutes) for toxic signs of systemic botulism in accordance with (IAW) Battelle SOP CORV-005. Criteria for initiation of 3,4-DAPP treatment (or placebo) will be animal-specific and will be determined through clinical severity scoring of select toxic signs (ptosis, muscular weakness, respiratory distress, oral/nasal discharge). A clinical severity score of 1 or greater in any one of the selected toxic signs will serve as a trigger to initiate 3,4-DAPP (or placebo) treatment.

3,4-DAPP treatment will be performed by the subcutaneous (SC) dosing route. Treatments will be delivered in bolus fashion. All dose volumes will not exceed 5 mL/kg. 3,4-DAPP treatment will be performed at a single dose level (4.12 mg/kg). Placebo (saline) dose volumes will be matched to the dose volume (mL/kg) employed for 3,4-DAPP treatment. Once an animal has triggered to receive SC treatments, repeat treatment frequency with 3,4-DAPP (or placebo) will be performed every 2 hours (± 15 minutes) until mortality or scheduled euthanasia at 4 days (96 hours ± 30 minutes) after the initial onset of a clinical severity score of 1 or greater in any one of the selected toxic signs. SC dose sites for treatment administration will be rotated throughout sites at the right and left hip/thigh area and between the scapulae. The total number of SC treatments will not exceed 47. The final treatment injection will not be performed immediately prior to scheduled euthanasia (96 hours ± 30 minutes after the onset of a clinical severity score of 1 or greater).

Previous studies have demonstrated that treatment with 3,4-DAP can reverse respiratory depression and restored mobility at terminal stages of botulism [7]. Because survival is the primary efficacy endpoint, animals that are judged to be moribund will not be euthanized. Survival will be defined as the number of animals alive or dead at 4 days (96 hours ± 30 minutes) after the onset of a clinical severity score of 1 or greater.

A summary of the Study Design is presented in Table 1. Detailed procedures for collected endpoints are presented below in subsequent sections (see Sections 10.3 through 10.8).

Table 1. Study Design

Group	Challenge; Dose; Dose Route; Dose Volume	Treatment; Dose; Dose Route; Dose Volume	Endpoints	# of Animals
1	BoNT/A1; 2.5x LD_{50}; IM; 0.0975 mL/kg	Saline; 0 mg/kg; SC; 2.06 mL/kg	Survival Clinical Observations	6
2		3,4-DAPP; 4.12 mg/kg; SC; 2.06 mL/kg		6

IM = intramuscular; SC = subcutaneous

9.1 ANIMAL RANDOMIZATION

Animals will be randomized to groups by weights collected prior to challenge activities (Day -3, Day -2, or Day-1). Randomization will be performed using the Provantis computer system in accordance with Battelle SOP Number COR.4-006 to ensure similar group mean weights. In the event of animal illness or injury prior to the start of the study, extra replacement animals will be assigned to groups by the Study Director.

9.2 BODY WEIGHTS

Animals will be weighed during quarantine and re-weighed as necessary for medical monitoring purposes. Animals will be weighed prior to the day of challenge for randomization to groups by body weight (Day -3, Day -2, or Day -1) and for challenge and treatment dose calculations. Additional weights may be taken at any time for medical monitoring purposes for any health or veterinarian related issues. Body weights will be collected in accordance with Battelle SOP COR.4-012 and will be recorded in Provantis in accordance with SOP COR.4-008.

9.3 ANIMAL OBSERVATIONS

9.3.1 Prior to Challenge

AM/PM clinical observations will be performed twice daily in accordance with Battelle SOP COR.5-005. A single AM observation will be made before challenge with BoNT/A1. Clinical observations will be recorded in Provantis in accordance with Battelle SOP COR.4-009.

9.3.2 Post-Challenge

A single PM observation will be performed on the day of challenge, after BoNT/A1 challenge. On Study Day 2, per the 24-hour clock, animals will be observed at 2-hour intervals (± 15

minutes) starting at 0800 and continuing at 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 0200, 0400, and 0600 through study termination at mortality or scheduled euthanasia. Clinical observations will be recorded in Provantis according to Battelle SOP Number COR.4-009. Paper documentation of observations may be performed if required.

9.3.3 Clinical Severity Scoring

At each clinical observation time-point, including post-treatment observations, clinical severity scoring will be performed IAW the Table 2 criteria. Paper documentation of clinical severity scores will be performed.

Table 2. Clinical Severity Scoring

Clinical Signs	Observation	Clinical Severity Score Assigned
Ptosis	Absent	0
	Slight	1
	Marked	2
Muscular Weakness	Normal	0
	Hunched Posture	1
	Prostrate but able to rise	2
	Prostrate but unable to rise	3
Respiratory Distress	Normal breathing	0
	Open mouth shallow (thoracic)	1
	Gasping and jerking (abdominal)	3
Oral Discharge	Absent	0
	Present	1
Nasal Discharge	Absent	0
	Present	1
Lethality	Alive	0
	Dead	10

10.0 ANIMAL CARE, HOUSING, AND ENVIRONMENTAL CONDITIONS

General procedures for animal care and housing will meet the AAALAC International recommendations, current requirements stated in the current "Guide for Care and Use of Laboratory Animals," current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act, as amended, and will conform to the testing facility SOPs. Prior to study initiation the protocol will be reviewed and approved by the Institutional Animals Care and Use Committee (IACUC).

10.1 RECEIPT, QUARANTINE, AND TRANSFER

Prior to transfer to this study, receipt and quarantine of animals under a Battelle IACUC-approved holding protocol will have been previously performed in accordance with Battelle SOPs. Previous testing of NHPs will be used to verify negative status for tuberculosis and to confirm that they are seronegative for Simian Immunodeficiency Virus (SIV), Simian T-Lymphotrophic Virus-I (STLV-1), and Macacine herpesvirus 1 (Herpes B virus), and negative for Simian Retrovirus (SRV1 & SRV2) by polymerase chain reaction (PCR).

Pole/collar and other training may be performed (Battelle SOP BBRC.VII-079) while animals remain in the holding protocol and will continue into the pre-challenge period after transfer to this study, as necessary. Prior to challenge, all animals will be acclimated to chair restraint IAW Battelle SOPs.

Animals will undergo a physical exam by a Battelle veterinarian prior to transfer. Only NHPs that are determined to be healthy by a Battelle clinical veterinarian will be transferred to the study protocol. Documentation of the transfer to this study will be retained in the study file. If any NHP is considered unacceptable for study purposes by a veterinarian or the Study Director, it will be removed from the study.

10.2 VETERINARY CARE

Animals will be observed by laboratory personnel prior to and following dosing. Animals that develop non-study-related illness or injury will be evaluated by a Battelle veterinarian for determination of treatment or disposition. In such cases, if in the opinion of the Study Director and a Battelle veterinarian, an animal is unfit for placement on study or in a moribund state prior to study the animal will be euthanized in accordance with Battelle SOPs.

10.3 PAIN AND DISTRESS

Discomfort and distress will be limited to that which is unavoidable in the conduct of scientifically valuable research. Early euthanasia will be performed for any moribund animals prior to challenge. After challenge, early euthanasia will not be performed as survival is the primary endpoint of efficacy (see Section 10.4 for further details).

10.4 CRITERIA FOR EUTHANASIA

Prior to challenge: Any animal judged to be moribund by a trained life sciences technician, Battelle veterinarian, or by the Study Director, will be euthanized.

Post-Challenge: As survival is the primary efficacy endpoint in this study, animals that are judged to be in a moribund state will not be euthanized. In previous nonhuman primate

BoNT/A1 studies, animals initially showing severe signs of BoNT intoxication have recovered to varying degrees following 3,4-DAPP treatment [8,9]. To prematurely terminate an animal that may otherwise survive would adversely alter statistical outcomes and overall interpretation of results. As a result, animals that appear to be moribund post-challenge will not be euthanized. Scheduled euthanasia will be performed for all animals surviving to 96 hours (± 30 minutes) after the onset of toxic signs associated with botulism (clinical severity score of greater than or equal to 1).

10.5 Animal Housing and Environmental Conditions

10.5.1 Housing

Animals may be socially housed (to the extent possible) prior to challenge. Following challenge, animals will be single housed. Single housing units will meet the requirements of the Guide for the Care and Use of Laboratory Animals (National Research Council 2011) and the Animal Welfare Act as amended, or as directed by a Battelle veterinarian and approved through Battelle's Institutional Animal Care and Use Committee. Socialization will be achieved by housing animals in the same room with visual, olfactory, and auditory contact.

10.5.2 Lighting

The light/dark cycle will be approximately 12 hours each per day using fluorescent lighting, in accordance with Battelle SOP BBRC.IV-002, with the exception of during study activities where lights may be on longer than 12 hours if necessary.

10.5.3 Temperature

Animal room temperatures will be controlled, monitored, and documented according to Battelle SOP BBRC.IV-008. Animal room temperature will be maintained at SOP defined criteria (64-84°F). Excursions will be evaluated by the Study Director to assess impact.

10.5.4 Humidity

The relative humidity of animal rooms will be maintained according to Battelle SOP BBRC.IV-008. The relative humidity of animal rooms will be maintained at SOP-defined criteria (30- 70%). Excursions will be evaluated by the Study Director to assess impact.

10.5.5 Air Handling

The air pressure and air changes of animal rooms will be maintained according to Battelle SOP COR.5-015.

10.5.6 Diet

NHPs will be fed Purina Certified Monkey Chow IAW Battelle SOP BBRC.VII-014. No contaminants that would affect the results of the study are known to be present in the feed.

Analytical reports of each lot of feed will be provided by the manufacturer. Analytical reports will be reviewed according to Battelle SOP COR.5-019 to ensure acceptable standards and freedom from levels of contaminants that may interfere with the purpose or conduct of the study.

10.5.7 Water

Cages will be equipped with an automatic watering system. Water will be supplied from the West Jefferson municipal water system and will be available ad libitum during the study. Water is analyzed at least once per year. Analysis is carried out IAW Battelle SOP BBRC. VIII-003. No contaminants that would affect the results of the study are known to be present in the water. Analytical results will be retained in facility records.

10.5.8 Enrichment

To promote and enhance the psychological well-being of the NHPs, both food and environmental enrichment will be provided as described in Battelle SOP COR.5-008. Documentation of the supplementation will be maintained in the study files.

11.0 COMPUTER SYSTEMS FOR DATA MANAGEMENT

The following computer systems may be used for data management:

Table 3. Data Acquisition Systems

Computer System*	Manufacturer	Data Type
ECMS	Siemens	Animal Facility Environmental
Insight, InfoCenter Suite, and APOGEE	Siemens	Building Monitoring (Room Temperatures, Light Cycles, Pressure, Humidity, Air Flow, Cold Storage Unit Temperature)
Provantis®	Instem	Clinical observations, body weights, randomizations
Sample Tracking System#	Battelle	Barcoded sample tracking for sample disposition and location
SAS®	SAS	Statistical Analysis
Stata	StataCorp, LLC	Statistical Analysis
Prism	GraphPad	Graphing

* Version numbers to be included in the final report.

\# Does not have archival capabilities but is otherwise validated as Part 11 compliant.

12.0 STATISTICAL ANALYSIS

To demonstrate a survival benefit with 3,4-DAPP treatment, animal numbers are based on the assumed outcomes of less than 1% survival in the vehicle treated animals (complete lethality)

and greater than 75% survival in 3,4-DAPP treated animals, with at least 90% power to detect a difference between groups.

The endpoint for survival will be defined by the number of animals in each group that are alive or dead at 96 hours (± 30 minutes) after the onset a clinical severity score of 1 or greater in any one of the selected toxic signs. Survival proportions with exact 95% confidence intervals will be calculated for each group. A two-sided Boschloo's test will be used to compare survival rates between groups, with the test performed at the 0.05 level. A log-rank test will be used to compare time to death after toxic sign onset (clinical severity of ≥ 1) between the two groups if the data are amenable. Time to death relative to challenge time will not be analyzed. A Kaplan Meier plot will be estimated relative to the time of toxic sign onset (clinical severity of ≥ 1). Additional statistical analyses may be performed at the direction of the Study Director. The final results of statistical analysis and all employed statistical methods will be provided as a Contributing Scientist Report for inclusion in the Final Report.

13.0 REPORTING

A draft report will be prepared and submitted to the Sponsor. The Sponsor shall submit comments on the draft report. Additional reviews and comments may be performed. A final report will be submitted to the Sponsor following receipt of the Sponsor's final comments.

14.0 STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

The disposition of study materials (data, documentation, samples, and/or specimens) is outlined in the following table.

Table 4. Storage of Study Materials

Study Material	Location or Disposition
Raw data from Battelle	Raw data will be archived at Battelle
Residual test article	Retention samples will not be archived. Residual test article will be retained for use on a future study.
Animals	NHPs used on study will be euthanized at the protocol-defined terminal time-point. Any unused extra animals may be euthanized, transferred to a training protocol, or transferred to another study.
Final Report	Report will be archived at Battelle

15.0 BIOSAFETY CONSIDERATIONS

A Battelle Environment, Safety, and Health Officer has been provided the opportunity to review this animal use protocol and all procedures required to execute this study.

Containment Level: The macaques will be housed in the ABSL-2 laboratory. Handling of BoNT/A1 will occur in BSL-2 laboratory.

Biohazard Safety: Personnel handling of macaques will wear appropriate personal protective equipment (PPE) as described in Battelle SOPs. All personnel working with BoNT/A1 will be performed following the Battelle BBRC Facility Safety Plan and Biosafety Manual (FSP) and other applicable policies and procedures.

Select Agent: The select agent utilized in this protocol is Botulinum neurotoxin serotype A1.

16.0 REFERENCES

References

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5. Kirsch, G.E, Narahashi, T. *3,4-diaminopyridine. A potent new potassium channel blocker*. Biophys J, 1978. 22(3):507-512.
6. Scott, A.B., and Suzuki, D. *Systemic Toxicity of Botulinum Toxin by Intramuscular Injection in the Monkey*. Movement Disorders, 1988. 3(4): 333-335.
7. Vazquez-Cintron E., Machamer J., Ondeck C., et. al. *Symptomatic treatment of botulism with a clinically approved small molecule*. JCI Insight, 2020. 5(2): e132891.
8. Beske, P.H. *Development of a Cynomolgus Monkey Model of Botulism for the Evaluation of Post-Symptomatic Medical Countermeasure Efficacy*. Final Report for Battelle Study 4454, 2019.
9. Beske, P.H. *Efficacy Evaluation of 3,4-Diaminopyridine to Acutely Reverse Toxicity in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1*. Battelle Study B05929 (report in preparation).

Standard Operating Procedures

1. Battelle SOP BBRC.IV-002, "Monitoring Room Lighting in Animal Rooms."
2. Battelle SOP BBRC.IV-008, "Monitoring Temperature and Humidity Conditions Using Automated HVAC Control and Monitoring Systems."
3. Battelle SOP BBRC.VII-006, "Animal Euthanasia at the Battelle Biomedical Research Center (BBRC)."
4. Battelle SOP BBRC.VII-014, "Care of Nonhuman Primates."
5. Battelle SOP BBRC.VII-026, "Receipt, Transfer, Quarantine, Monitoring, and Release of Experimental Animals."
6. Battelle SOP BBRC.VII-079, "Acclimation of Nonhuman Primates to Pole and Collar Restraint."
7. Battelle SOP BBRC.VIII-003, "Supplying Water and Monitoring Water Quality of the Manual and Automatic Watering Systems."
8. Battelle SOP BBRC.VIII-010, "Operating the Pathological Incinerator."
9. Battelle SOP BBRC.X-014, "Detection, Quantitation and Optimal Dose Calibration of Botulinum Toxins and Antitoxins (Antibodies) Using the Mouse Bioassay."
10. Battelle SOP COR.1-002, "Operation of Archives."
11. Battelle SOP COR.4-006, "Randomization and Animal Management in the Provantis Computer System."
12. Battelle SOP COR.4-007, "Defining In-Life Data Entry Sessions in the Provantis Computer System."
13. Battelle SOP COR.4 -008, "Entering Body Weights, Recording Generalized Results, and Marking Animals Dead in the Provantis Computer System."
14. Battelle SOP COR.4-009, "Entering Clinical Observations in the Provantis Computer System."
15. Battelle SOP COR.5-005, "Conducting Clinical Observations."

16. Battelle SOP COR.5-008, "Environmental Enrichment of Nonhuman Primates."

17. Battelle SOP COR.5-009, "Injections."

18. Battelle SOP COR.5-012, "Measuring Body Weight, Food Consumption, and Water Consumption."

19. Battelle SOP COR.5-015, "HVAC Systems in Animal Resources."

20. Battelle SOP COR.5-019, "Feed Source, Storage, and Handling."

Appendix B Summary of Deviations

SUMMARY OF DEVIATIONS

Deviations from the study protocol are summarized below; SOP deviations are maintained in the study file

Deviation Number	Date Occurred	Description	Impact
DR-B06188-BBRC-0001	4/14/2022	Per Section 9.0 Experimental Design of protocol B06188, "SC dose sites for treatment administration will be rotated throughout the sites at the right and left hip/thigh area and between the scapulae. Due to logistical and safety reasons, starting with 1200 treatments on 4/14/22 and continuing through the end of the study, the hip/thigh dose site is being removed and all treatments will be given in the right and left scapula areas only.	The elimination of the hip/thigh area improves the safety, accuracy and efficiency of the treatment administrations. Due to the small size of the NHP's there is a very small amount of skin surrounding the hip/thigh area. Initial treatment administrations, although successful and proper, showed some difficulty in restraining the animal's legs to prevent them from sudden movements, moving when stuck with the needle, and in locating an adequate amount of skin in that area to "tent up" to administer the dose. Safety concerns surrounding the high potential for the doser to be accidentally stuck with the needle from the animal twitching and moving as well as the potential for an inaccurate dose volume to be received due to the animal moving and twitching as the plunger is being pushed, supported a change to remove the hip/thigh area as a treatment area. Use of the scapula area is a much safer, easier and controlled location for administering the treatments. This area was previously used successfully for executing Study B05929. The treatment forms were updated to revise the documented treatment sites.

Appendix C Challenge Article COA

METABIOLOGICS, INC
505 S. ROSA RD, SUITE 18
MADISON WI 53719
(608) 441-2730 fax (608) 441-2731

Certificate of Analysis

Description: Clostridium botulinum Complex Type A

Lot No. #: A081508-01

Volume of toxin shipped : 5.0 ml 25 mg Total

Concentration of toxin: 5.0 mg/ml

Specific Activity: 3.5 X 10^7 LD50/mg

The toxin was shipped in 0.05 M Sodium Phosphate + 0.2 M sodium chloride buffer, pH 6.2. Toxin was passed through a 0.22 micron syringe filter.

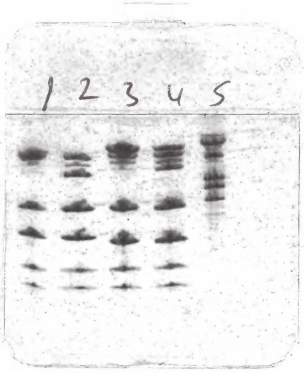
The toxin described above was tested for specific activity by mouse bioassay, identity by type specific antibody neutralization and purity by SDS-PAGE.

Lot number A081508-01 of Type A toxin complex has been released.

Released by: [redacted]

Date: 8/15/08

Storage conditions: Refrigerate (2-8 C) short term or freeze.
Expires 8/15/09



SDS-PAGE

1 A complex
2 A reduced
3 B complex
4 B reduced
5 HMW Markers

Appendix D Test Article and Vehicle

Patheon
A HEALTHIER WORLD. DELIVERED.

Patheon API Manufacturing
309 Delaware Street
Greenville, SC 29605
864-299-8600
Patheon.com

CERTIFICATE OF ANALYSIS

Project Code: CAY528

Product Name: Amifampridine Phosphate Drug Substance

Inventory Code: PR-PRD-CAY01

Lot Number: P528FPM-19-002

Manufacturing Date: 12/16/2019

Date of Analysis: 03/11/2020

TEST	SPECIFICATION		RESULTS	
Description	White Crystalline Powder		White Crystalline Powder	
Identification (FTIR)	Conforms to reference		Conforms to reference	
Identification (Phosphate)	Conforms		Conforms	
Assay (HPLC) on dried basis	98.0 – 102.0%		99.2%	
Related Substances	4-aminopyridine (AMPY, 4-AP)	≤ 0.05%	4-aminopyridine (AMPY, 4-AP)	< 0.05%
	3-nitro-4-aminopyridine (ANPB)	≤ 0.05%	3-nitro-4-aminopyridine <0.05%(ANPB)	< 0.05%
	Individual Unidentified	≤ 0.10%	Individual Unidentified	< 0.05%
	Total	≤ 0.50%	Total	< 0.50%
Residual Solvents	Ethanol	NMT 3000 ppm	Ethanol	< 397 ppm
	1,2-Dichloroethane	NMT 5 ppm	1,2-Dichloroethane	< 2 ppm
	Triethylamine	NMT 125 ppm	Triethylamine	< 58 ppm
3-nitro-4-pyridinyl-ether ester	NMT 0.001%		ND (<0.001%)	
Clarity and color of solution	Clear, colorless solution		Clear, colorless solution	

Patheon API Manufacturing
309 Delaware Street
Greenville, SC 29605
864-299-8600
Patheon.com

CERTIFICATE OF ANALYSIS

TEST	SPECIFICATON	RESULTS
Solubility	Easily soluble in Water	Easily soluble in Water
Melting Point (ID)	225-231°C	229°C
pH (ID), 1% (w/v) in water	4.2 - 4.6	4.5
Loss on Drying	NMT 1.0%	0.3%
Palladium	NMT 15 ppm	< 5 ppm
Water Content (KF)	NMT 1.0%	<0.05% (0.02%)
Particle Size	D(v, 0.9) ≤ 50 µm	37 µm
Total aerobic count	NMT 1000 CFU/g	< 10 CFU/g
Yeast and mold (Fungi)	NMT 100 CFU/g	< 10 CFU/g
Absence of E. Coli	Absent in 1 g	Absent in 1 g

Storage: Store in tight containers at warehouse conditions

Re-test date: 12/2022

Record of Revision

Version:	Effective Date:	Reason:
1	JUN 08 2020	New

Patheon
A HEALTHIER WORLD. DELIVERED.

Patheon API Manufacturing
309 Delaware Street
Greenville, SC 29605
864-299-8600
Patheon.com

CERTIFICATE OF ANALYSIS

This lot was manufactured in accordance with the requirements for production of Active Pharmaceutical Ingredients (APIs) as outlined in ICH Q7 "Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients".

[redacted]

05/29/2020
Date

05/29/2020
Date

NOVA-TECH
ANIMAL HEALTH

CERTIFICATE OF ANALYSIS

PRODUCT:	Sterile Saline
CAT. #:	C1880750
LOT #:	A2110132
MANUFACTURE DATE:	October 19, 2021
EXPIRATION DATE:	OCT 2024

TEST	SPECIFICATION	RESULT
Sterility	Sterile	Sterile
Volume	≥ 500 mL	> 500 mL
Appearance	Clear, colorless	Pass
pH	4.5 - 7.0	6.3
Sodium Chloride Assay	855 - 945 mg/dL	894 mg/dL
Chloride Identification	Pass	Pass
Sodium Identification (Method I)	Pass	Pass
Sodium Identification (Method II)	Pass	Pass
Heavy Metals	≤ 0.001% w/v	Pass
Iron Test	≤ 2 ppm	Pass
Bacterial Endotoxin	≤ 0.5 EU/mL	< 0.025 EU/mL
Particulates ≥ 10 µm	≤ 25 counts/mL	Pass
Particulates ≥ 25 µm	≤ 3 counts/mL	Pass

This product meets specifications and is eligible for release.

[redacted]

NOVA-TECH
ANIMAL HEALTH

CERTIFICATE OF ANALYSIS

PRODUCT:	Sterile Saline
CAT. #:	C1880750
LOT #:	A2110132
MANUFACTURE DATE:	October 19, 2021
EXPIRATION DATE:	OCT 2024

TEST	SPECIFICATION	RESULT
Sterility	Sterile	Sterile
Volume	≥ 500 mL	> 500 mL
Appearance	Clear, colorless	Pass
pH	4.5 - 7.0	6.3
Sodium Chloride Assay	855 - 945 mg/dL	894 mg/dL
Chloride Identification	Pass	Pass
Sodium Identification (Method I)	Pass	Pass
Sodium Identification (Method II)	Pass	Pass
Heavy Metals	≤ 0.001% w/v	Pass
Iron Test	≤ 2 ppm	Pass
Bacterial Endotoxin	≤ 0.5 EU/mL	< 0.025 EU/mL
Particulates ≥ 10 µm	≤ 25 counts/mL	Pass
Particulates ≥ 25 µm	≤ 3 counts/mL	Pass

This product meets specifications and is eligible for release.

[redacted]

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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REVW:	05/05/2020	**EFFC:**	06/15/2020	**SUBTYPE:**	7	**AREA:**	T
DESC:	Certificate of Analysis Hospira, Inc. (Rocky Mount N.C.) CQ						
Written By/Date:	[redacted]			**FE:**	None	**UNIT:**	FCF/PF/Rocky Mount
Approved By/Date:	[redacted]		6-8-20	[redacted]			6-8-20

Specification No.:	60.07983ALLCODE

Technical Note:

1. This product has been manufactured and tested in current good manufacturing practices (CGMP) facilities in accordance with appropriate regulations. This product meets applicable specifications, applicable regulatory submissions or marketing authorizations and, where appropriate, compendial requirements. The undersigned certifies this to be a true representation of the results.
2. Pfizer list 079830424 corresponds to ICU List 079830416
3. Pfizer list 079830464 corresponds to ICU List 079830466
4. Pfizer list 079830473 corresponds to ICU List 079830456
5. Pfizer list 079830489 corresponds to ICU List 079830450

Product Manufacturing Information

Date of Manufacture: 13 Dec 2020 Batch Size 129000L NDC No.: 0990798303

Final Released Accountability: 220224 Units 9176 Cases

STEP/PROCEDURE	DOC. REF.	SIGNATURE/DATE
Manufacturing Formula	92.D-7983 Current Date: 01-30-14	
Commodity and Process Summary	35. 079830464 Current Date: 06-13-19	
Printed Material Summary	40.0798304AC [redacted] 12-09-20	
Sampling and Testing Requirements	60.07983ALLCODE Current Date: 08-20-19	

[redacted]
Enter By/Date

13 Dec 2020
Dates Manufactured From _____ To 15 Dec 2020

Water For Injection, USP - Pass ✓ Fail _____

Signature/Date [redacted]

7983-04-64
0.9% SODIUM CHLORIDE INJ., USP

24-701-FW

EXP. DATE 1DEC2022

Comments:

PERRYHM 11/25/20

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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PRODUCT TEST RESULTS

STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
Batch Release Systems I. Physical Requirements: A. Clarity. 1. Solution must be clear. 2. Solution must not contain one or more particles which are visible upon attentive examination.	90.P-0416	Pass ✓ Fail___	
B. Volume (250 mL) 250 to 300 mL (500 mL) 500 to 560 mL (1000 mL) 1000 to 1055 mL	90.P-0759	538 mL Average	
C. Sterility Must meet Requirements of Parametric release.	90.M-0476 (04-44,04-49) 90.M-0477 (All other codes)	Pass ✓ Fail___	
D. QAR Yes ( ) No (✓) Check Applicable Box QAR Number_[redacted] QAR Number_ QAR Number_ QAR Number_			

[redacted]
BRQ REVIEW

7983-04-64
0.9% SODIUM CHLORIDE INJ., USP

24-701-FW

EXP. DATE 1DEC2022

Comments:

PERRYHM 11/25/20

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
II. Chemical Requirements:			
A. Sodium Chloride Final product limits 95.0% to 105.0%	90.C-0042	97.5 %	[redacted]
B. Elemental Impurities	90.C-2377	Blank not used Pass ✓ Fail ___	
Hg NMT 0.75 ng/mL		LT 0.38 ng/mL	
Cd NMT 1.0 ng/mL		LT 0.50 ng/mL	
Pb NMT 2.5 ng/mL		None Detected ng/mL	
Co NMT 2.5 ng/mL		LT 1.25 ng/mL	
Tl NMT 4 ng/mL		LT 2.00 ng/mL	
Ir NMT 5 ng/mL		None Detected ng/mL	
Os NMT 5 ng/mL		LT 2.50 ng/mL	
Pt NMT 5 ng/mL		None Detected ng/mL	
Pd NMT 5 ng/mL		None Detected ng/mL	

[redacted]

01-14-2021

7983-04-64
0.9% SODIUM CHLORIDE INJ., USP

24-701-FW

EXP. DATE 1DEC2022

Comments:

PERRYHM 11/25/20

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
Rh NMT 5 ng/mL		LT 2.50 ng/mL	
Ru NMT 5 ng/mL		LT 2.50 ng/mL	
V NMT 5 ng/mL		None Detected ng/mL	
As NMT 7.5 ng/mL		None Detected ng/mL	
Ag NMT 5 ng/mL		LT 2.50 ng/mL	
Ni NMT 10 ng/mL		None Detected ng/mL	
Mo NMT 45 ng/mL		LT 20.0 ng/mL	
Se NMT 40 ng/mL		LT 20.0 ng/mL	
Au NMT 50 ng/mL		LT 25.0 ng/mL	
Cu NMT 65 ng/mL		LT 25.0 ng/mL	
Li NMT 125 ng/mL		None Detected ng/mL	
Sb NMT 45 ng/mL		LT 20.0 ng/mL	
Sn NMT 300 ng/mL		None Detected ng/mL	
Cr NMT 550 ng/mL		LT 25.0 ng/mL	

[redacted] -2021

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments:

PERRYHM 11/25/20

Hospira Inc, a Pfizer Company
Version # 7.0 DOC # W7983CQA

STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
Ba NMT 350 ng/mL		LT 25.0 ng/mL	
Zn NMT 650 ng/mL		LT 163 ng/mL	
Mn NMT 125 ng/mL		None detected ng/mL	
Fe NMT 650 ng/mL		None detected ng/mL	

[redacted]

01-14-2021

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments: PERRYHM 11/25/20

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
C. Iron Final product limits: Not more than 0.0002% (2 ppm)	90.C-0869	NMT 0.0002% (2ppm)	[redacted]
D. Identification Responds to test for:			[redacted]
Sodium	90.C-1648	Pass ✓ Fail___	
Chloride	90.C-0003	Pass ✓ Fail___	
E. pH Final product limits: 4.5 and 7.0	90.C-0021	6.0	[redacted]
F. Residual Solvents Meets USP <467>	N/A	No Class 1, Class 2, Class 3 or other solvents used. Drug product testing is not required.	
			(1) CQ REVIEW

60.Document Exceptions:
Please list all exceptions to the 60.document, related tests, the date these tests were initiated, and the test results.

Document #	Related Test	Date Test Initialized	Test Results
		[redacted]	

STEP/PROCEDURE	SIGNATURE/DATE
The undersigned certifies that the information contained herein is accurate and complete, and the release for distribution was approved.	[redacted] 01-14-2021 QUALITY HOSPIRA, INC. ROCKY MOUNT, NC

(1) Data transcribed from glims before disposition by QA Auditor/Approver. [redacted]

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments: PERRYHM 11/25/20

Version # 7.0

Hospira Inc, a Pfizer Company
DOC # W7983CQA

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Note: Once product is released, email a copy of the completed Certificate of Analysis to:

DL-Distribution-Quality@icumed.com

[redacted]
Sent By

01-14-2021
Date

END OF DOCUMENT

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments:

PERRYHM 11/25/20

Version # 5.0

Hospira Inc, a Pfizer Company
DOC # W7983BQAC

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REVW:	04/05/2013	**EFFC:**	08/26/2013	**SUBTYPE:**	8	**AREA:**	B
DESC:	Certificate of Analysis Hospira, Inc. (Rocky Mount N.C.) BQ						
Written By/Date:	[redacted]			**FE:**	None	**UNIT:**	FCF/PF/Rocky Mount
Approved By/Date:	[redacted]		8-19-13				

Specification No.:	60.07983ALLCODE, QPO.18.004

Technical Note:
1. This product has been manufactured and tested in current good manufacturing practices (CGMP) facilities in accordance with appropriate regulations. This product meets applicable specifications, applicable regulatory submissions or marketing authorizations and, where appropriate, compendial requirements. The undersigned certifies this to be a true representation of the results.

Product Test Results

STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
I. Physical Requirements A. Particulate Matter 1. Must meet test requirements.	90.P-0452 LIMS	Pass ✓ Fail ___	
2. Report the numerical results found.	Generated	≥ 10UM / ≥ 25UM 1 / 0 0 / 0 1 / 0 0 / 0 0 / 0 0 / 0 1 / 0 1 / 0 0 / 0 1 / 0	[redacted] 01-14-2021 TRANSCRIBED BY
II. Biological Requirements A. Bacterial Endotoxin Not more than 0.50 EU/ML	90.B-0813 LIMS Generated	LT 0.25 EU/ML	[redacted] 01-14-2021
B. Solution Bioburden Must meet requirements	QPO.29.001 WGENVIRO	Pass ✓ Fail ___	[redacted] BQ REVIEW

END OF DOCUMENT

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments:

PERRYHM 11/25/20

Version # 5.0

Hospira Inc, a Pfizer Company
DOC # WGFCFMQAIN

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REVW:	03/17/2020	**EFFC:**	08/25/2020	**SUBTYPE:**	9	**AREA:**	Q
DESC:	Manufacturing Quality Index Sheet						
Written By/Date:	[redacted]			**FE:**	None	**UNIT:**	FCF/Rocky Mount
Approved By/Date:	[redacted]		7-31-20				

Specification and SOP No.:	QCP.05.001, BFCF_MQA_WOAUD00.EWI

Technical Notes: None

Lab Data Checklist:

Biological (BQ)	Yes	N/A
Chemistry (CQ)	Yes	N/A

I verified that the above packages are present in the batch record.

[redacted] 4 Jan 2021
Batch Release Specialist/Designee

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments: PERRYHM 11/25/20

Version # 5.0

Hospira Inc, a Pfizer Company
DOC # WGFCFMQAIN

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Technical Notes:

The Batch Record Specialist/Designee must complete the checklist below to ensure that all packages within each batch record are inclusive/present and have been audited prior to final release. N/A indicates that the package is not required.

Batch Record Package Checklist:

Solution	Yes	N/A
Equipment	Yes	N/A
Commodity	Yes	N/A
Filling	Yes	N/A
R2 Labeling	Yes	N/A
Label Room	Yes	N/A
Finishing	Yes	N/A
Overwrap	Yes	N/A
Fabrication	Yes	N/A
Sterilizer	Yes	N/A

I have verified that the above packages have been audited and are present in the batch record.

[redacted] 4 Jan 2021

① Change date format [redacted]

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments: PERRYHM 11/25/20

Version # 5.0

Hospira Inc, a Pfizer Company
DOC # WGFCFMQAIN

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STEP/PROCEDURE	REF. DOC.	EXCEPTION DOCUMENTS	ENTERED BY DATE
1. If batch record requires exception documents, then exception document numbers are:	S6100_0070 S6100_0203	5480007 ② / N/A	( ) N/A ② wrong number
2. Is this package a Master/Reference batch record? YES (NO) (circle one) If yes then all Master/Reference requirements must be met.	S6116_0357	[redacted]	[redacted]
3. I certify that the following batch record has met all specifications relevant to the production, control, and release of this lot.			[redacted] FINAL BATCH RECORD REVIEW

Final Approval Release Information

Matitm/List ① N/A Batch Record Acc. No. ① N/A

Manufacturing Department: ( ) FCF-0580 ( ) DCIC-0504

Entry Code: ① N/A ( ) 307 ① N/A ( ) 309-DCIC

Load	Quantity	Load	Quantity	Load	Quantity
① N/A	9176 ② N/A			① N/A	

① N/A's not needed [redacted] 01-14-21

Check Appropriate Box: (✓) Final Release ( ) Partial Release
( ) Lot Rejection

Material Status Change By: [redacted] Date: 01-14-2021

END OF DOCUMENT

Expiration date of 12012022 verified to be correct for Lot 24-701-FW [redacted]

7983-04-64 24-701-FW EXP. DATE 1DEC2022
0.9% SODIUM CHLORIDE INJ., USP

Comments: PERRYHM 11/25/20

Appendix E Treatment Times

Study B06188
Treatment Times

Group	Animal ID	IM Challenge	SC Treatment*	Treatment Dates and Time								
				1st Treatment (Date & Time)	2nd Treatment (Date & Time)	3rd Treatment (Date & Time)	4th Treatment (Date & Time)	5th Treatment (Date & Time)	6th Treatment (Date & Time)	7th Treatment (Date & Time)	8th Treatment (Date & Time)	9th Treatment (Date & Time)
1	BM813G	BoNT/A1	saline (0 mg/kg)	4/13/2022 20:30	4/13/2022 22:30	4/14/2022 00:28	4/14/2022 02:23	4/14/2022 04:22	4/14/2022 06:23	4/14/2022 08:28	4/14/2022 10:22	4/14/2022 12:22
	SA179G			4/13/2022 20:18	4/13/2022 22:19	4/14/2022 00:21	4/14/2022 02:17	4/14/2022 04:15	4/14/2022 06:16	4/14/2022 08:21	4/14/2022 10:13	4/14/2022 12:17
	BM842C			4/13/2022 20:24	4/13/2022 22:23	4/14/2022 00:24	4/14/2022 02:19	4/14/2022 04:18	4/14/2022 06:19	4/14/2022 08:24	4/14/2022 10:15	4/14/2022 12:19
	JR81CB			4/13/2022 14:15	4/13/2022 16:11	4/13/2022 18:12	4/13/2022 20:10	4/13/2022 22:13	4/14/2022 00:15	4/14/2022 02:10	4/14/2022 04:08	4/14/2022 06:10
	BC114A			4/13/2022 20:27	4/13/2022 22:26	4/14/2022 00:26	4/14/2022 02:21	4/14/2022 04:19	4/14/2022 06:21	4/14/2022 08:26	4/14/2022 10:20	4/14/2022 12:21
	AL403H			4/13/2022 22:33	4/14/2022 00:31	4/14/2022 02:25	4/14/2022 04:24	4/14/2022 06:25	4/14/2022 08:30	4/14/2022 10:25	4/14/2022 12:24	4/14/2022 14:36
2	GA669H	BoNT/A1	3,4-DAPP (4.12 mg/kg)	4/14/2022 00:46	4/14/2022 02:36	4/14/2022 04:34	4/14/2022 06:34	4/14/2022 08:39	4/14/202210:34	4/14/2022 12:34	4/14/2022 14:46	4/14/2022 16:42
	AR787L			4/14/2022 04:46	4/14/2022 06:36	4/14/2022 08:41	4/14/2022 10:35	4/14/2022 12:36	4/14/2022 14:48	4/14/2022 16:44	4/14/2022 18:50	4/14/2022 20:32
	SL42B			4/14/2022 00:43	4/14/2022 02:33	4/14/2022 04:32	4/14/2022 06:32	4/14/2022 08:37	4/14/2022 10:31	4/14/2022 12:33	4/14/2022 14:43	4/14/2022 16:40
	AK950J			4/14/2022 00:40	4/14/2022 02:30	4/14/2022 04:30	4/14/2022 06:30	4/14/2022 08:34	4/14/2022 10:29	4/14/2022 12:31	4/14/2022 14:40	4/14/2022 16:37
	AK394MB			4/13/2022 14:18	4/13/2022 16:18	4/13/2022 18:15	4/13/2022 20:12	4/13/2022 22:16	4/14/2022 00:18	4/14/2022 02:14	4/14/2022 04:12	4/14/2022 06:13
	BM162L			4/13/2022 22:37	4/14/2022 00:34	4/14/2022 02:28	4/14/2022 04:27	4/14/2022 06:27	4/14/2022 08:33	4/14/2022 10:27	4/14/2022 12:29	4/14/2022 14:38

Group	Animal ID	IM Challenge	SC Treatment*	Treatment Dates and Time								
				10thTreatment (Date & Time)	11th Treatment (Date & Time)	12th Treatment (Date & Time)	13th Treatment (Date & Time)	14th Treatment (Date & Time)	15th Treatment (Date & Time)	16th Treatment (Date & Time)	17th Treatment (Date & Time)	18th Treatment (Date & Time)
1	BM813G	BoNT/A1	saline (0 mg/kg)	4/14/2022 14:34	4/14/2022 16:29	4/14/2022 18:33	4/14/2022 20:21	4/14/2022 22:22	4/15/2022 00:20	4/15/2022 02:07	4/15/2022 04:00	4/15/2022 06:24
	SA179G			4/14/2022 14:26	4/14/2022 16:23	4/14/2022 18:27	4/14/2022 20:16	4/14/2022 22:19	4/15/2022 00:13	4/15/2022 02:03	4/15/2022 03:56	4/15/2022 06:19
	BM842C			4/14/2022 14:28	4/14/2022 16:24	4/14/2022 18:29	4/14/2022 20:18	4/14/2022 22:20	4/15/2022 00:17	4/15/2022 02:05	4/15/2022 03:58	4/15/2022 06:22
	JR81CB			4/14/2022 08:16	4/14/2022 10:10	4/14/2022 12:13	4/14/2022 14:21	4/14/2022 16:18	4/14/2022 18:21	4/14/2022 20:13	4/14/2022 22:15	4/15/2022 00:03
	BC114A			4/14/2022 14:30	4/14/2022 16:26	4/14/2022 18:32	4/14/2022 20:19					
	AL403H			4/14/2022 16:31	4/14/2022 18:36	4/14/2022 20:22	4/14/2022 22:24	4/15/2022 00:22	4/15/2022 02:09	4/15/2022 04:01		
2	GA669H	BoNT/A1	3,4-DAPP (4.12 mg/kg)	4/14/2022 18:48	4/14/2022 20:31	4/14/2022 22:31	4/15/2022 00:34	4/15/2022 02:19	4/15/2022 04:09	4/15/2022 06:43		
	AR787L			4/14/2022 22:33	4/15/2022 00:37	4/15/2022 02:22	4/15/2022 04:10	4/15/2022 06:45	4/15/2022 08:33	4/15/2022 10:31	4/15/2022 12:31	4/15/2022 14:35
	SL42B			4/14/2022 18:45	4/14/2022 20:29	4/14/2022 22:29	4/15/2022 00:32	4/15/2022 02:17	4/15/2022 04:07	4/15/2022 06:41	4/15/2022 08:30	4/15/2022 10:28
	AK950J			4/14/2022 18:42	4/14/2022 20:26	4/14/2022 22:28	4/15/2022 00:29	4/15/2022 02:14	4/15/2022 04:05	4/15/2022 06:39	4/15/2022 08:28	4/15/2022 10:26
	AK394MB			4/14/2022 08:20	4/14/2022 10:12	4/14/2022 12:14	4/14/2022 14:24	4/14/2022 16:21	4/14/2022 18:24	4/14/2022 20:15	4/14/2022 22:17	4/15/2022 00:10
	BM162L			4/14/2022 16:34	4/14/2022 18:39	4/14/2022 20:24	4/14/2022 22:26	4/15/2022 00:26	4/15/2022 02:12	4/15/2022 04:04	4/15/2022 06:33	4/15/2022 08:24

Group	Animal ID	IM Challenge	SC Treatment*	Treatment Dates and Time								
				19thTreatment (Date & Time)	20th Treatment (Date & Time)	21st Treatment (Date & Time)	22nd Treatment (Date & Time)	23rd Treatment (Date & Time)	24th Treatment (Date & Time)	25th Treatment (Date & Time)	26th Treatment (Date & Time)	27th Treatment (Date & Time)
1	BM813G	BoNT/A1	saline (0 mg/kg)	4/15/2022 08:21	4/15/2022 10:17	4/15/2022 12:15	4/15/2022 14:15	4/15/2022 16:15	4/15/2022 18:16	4/15/2022 20:15	4/15/2022 22:20	
	SA179G											
	BM842C			4/15/2022 08:19								
	JR81CB											
	BC114A											
	AL403H											
2	GA669H	BoNT/A1	3,4-DAPP (4.12 mg/kg)									
	AR787L			4/15/2022 16:33	4/15/2022 18:34	4/15/2022 20:32	4/15/2022 22:32	4/16/2022 00:35				
	SL42B			4/15/2022 12:28	4/15/2022 14:33	4/15/2022 16:31	4/15/2022 18:33	4/15/2022 20:31	4/15/2022 22:29	4/16/2022 00:33		
	AK950J			4/15/2022 12:25	4/15/2022 14:26	4/15/2022 16:25	4/15/2022 18:26	4/15/2022 20:26	4/15/2022 22:26	4/16/2022 00:29	4/16/2022 02:29	4/16/2022 04:25
	AK394MB			4/15/2022 02:01	4/15/2022 03:55	4/15/2022 06:16	4/15/2022 08:16	4/15/2022 10:12	4/15/2022 12:08	4/15/2022 14:04	4/15/2022 16:04	4/15/2022 18:04
	BM162L			4/15/2022 10:23	4/15/2022 12:22	4/15/2022 14:24	4/15/2022 16:22	4/15/2022 18:25	4/15/2022 20:24	4/15/2022 22:24	4/16/2022 00:27	4/16/2022 02:27

Group	Animal ID	IM Challenge	SC Treatment*	Treatment Dates and Time									
				28thTreatment (Date & Time)	29th Treatment (Date & Time)	30th Treatment (Date & Time)	31st Treatment (Date & Time)	32nd Treatment (Date & Time)	33rd Treatment (Date & Time)	34th Treatment (Date & Time)	35th Treatment (Date & Time)	36th Treatment (Date & Time)	37th Treatment (Date & Time)
1	BM813G	BoNT/A1	saline (0 mg/kg)										
	SA179G												
	BM842C												
	JR81CB												
	BC114A												
	AL403H												
2	GA669H	BoNT/A1	3,4-DAPP (4.12 mg/kg)										
	AR787L												
	SL42B												
	AK950J												
	AK394MB			4/15/2022 20:05	4/15/2022 22:18	4/16/2022 00:17	4/16/2022 02:25	4/16/2022 04:13	4/16/2022 06:06	4/16/2022 08:08	4/16/2022 10:05	4/16/2022 12:05	
	BM162L			4/16/2022 04:22	4/16/2022 06:23	4/16/2022 08:22	4/16/2022 10:22	4/16/2022 12:22	4/16/2022 14:25	4/16/2022 16:22	4/16/2022 18:22	4/16/2022 20:22	4/16/2022 22:22

Prepared by: AV 7.5.22

QC Review by: JDW 7/6/22

Appendix F Mortality

Study B06188
Mortality

Group	Animal ID	Weight	IM Challenge	SC Treatment	Challenge (Date & Time)	First Observation of Toxicity (Date & Time)	Trigger Criteria Met for Subsequent Treatments (Date & Time)[#]	Time of Death (Date & Time)	Time-to-First Observation of Toxicity after Challenge (hrs:min)	Time-to-Trigger Criteria after Challenge (hrs:min)[#]	Time-to-Death after Challenge (hrs:min)	Time-to-Death after First Observation of Toxicity (hrs:min)	Time-to-Death after Trigger Criteria (hrs:min)[#]
1	BM813G	2.84	BoNT/A1 (97.5 $MIPLD_{50}$/kg)	saline (0 mg/kg)	4/11/2022 8:57	4/13/2022 20:01	4/13/2022 20:01	4/16/2022 0:09	59:04	59:04	111:12	52:08	52:08
	SA179G	2.97			4/11/2022 8:25	4/13/2022 19:59	4/13/2022 19:59	4/15/2022 6:54	59:34	59:34	94:29	34:55	34:55
	BM842C	3.27			4/11/2022 8:28	4/13/2022 20:00	4/13/2022 20:00	4/15/2022 10:03	59:32	59:32	97:35	38:03	38:03
	JR81CB	3.48			4/11/2022 8:48	4/13/2022 14:02	4/13/2022 14:02	4/15/2022 1:52	53:14	53:14	89:04	35:50	35:50
	BC114A	3.67			4/11/2022 8:54	4/13/2022 20:00	4/13/2022 20:00	4/14/2022 22:08	59:06	59:06	85:14	26:08	26:08
	AL403H	4.21			4/11/2022 8:12	4/13/2022 22:00	4/13/2022 22:00	4/15/2022 6:29	61:48	61:48	94:17	32:29	32:29
2	GA669H	3.16	BoNT/A1 (97.5 $MIPLD_{50}$/kg)	3,4-DAPP (4.12 mg/kg)	4/11/2022 8:36	4/14/2022 0:09	4/14/2022 0:09	4/15/2022 6:54	63:33	63:33	94:18	30:45	30:45
	AR787L	3.21			4/11/2022 8:43	4/14/2022 4:10	4/14/2022 4:10	4/16/2022 2:14	67:27	67:27	113:31	46:04	46:04
	SL42B	3.46			4/11/2022 8:40	4/14/2022 0:06	4/14/2022 0:06	4/16/2022 2:08	63:26	63:26	113:28	50:02	50:02
	AK950J	3.57			4/11/2022 8:32	4/14/2022 0:04	4/14/2022 0:04	4/16/2022 6:26	63:32	63:32	117:54	54:22	54:22
	AK394MB	3.84			4/11/2022 8:15	4/13/2022 14:08	4/13/2022 14:08	4/16/2022 14:28	53:53	53:53	126:13	72:20	72:20
	BM162L	4.37			4/11/2022 8:19	4/13/2022 22:04	4/13/2022 22:04	4/16/2022 23:58	61:45	61:45	135:39	73:54	73:54

[#] trigger criteria defined as Clinical Severity Score ≥ 1; hrs:min = hours:minutes; IM = intramuscular; $MIPLD_{50}$ = mouse interperitoneal LD_{50}; SC = subcutaneous

Prepard by: MV 5.16.22
QC Review by: JDV 5/16/22

Appendix G Clinical Observations with Clinical Severity Scores

Study B06188 Clinical Observations

Observation Key			
N=Normal	A = ataxia	LB = labored respirations	LR =lateralrecumbency
D=diarrhea	P = ptosis	SR = shallow respirations	PR = prostrate
NS = no stool	L = lethargic	DR = decreasedrespirations	FD = founddead
SS = Soft stool	OD = oraldischarge	IR = increasedrespirations	
V = vomiting	ND = nasaldischarge	FAR = forced abdominal respirations	
	H = hunchedposture	G = gasping	
( ) = clinical score		= Treatment Trigger	TCS =Total Clinical Severity Score

Clinical Scoring Table		
Clinical Sign	**Observation**	**Clinical Severity Score Assigned**
Ptosis	Absent	0
	Slight	1
	Marked	2
Muscular Weakness	Normal	0
	Hunched Posture	1
	Prostrate -able to rise	2
	Prostrate -unable to rise	3
Respiratory Distress	Normal Breathing	0
	Open mouth shallow breathi ng/gh t gasping (thoracic)	1
	Strong Gasping and Jerking (Forced abdominal breathing)	3
Oral Discharge	Absent	0
	Present	1
Nasal Discharge	Absent	0
	Present	1
Lethality	Alive	0
	Dead	10

				Study Day 1 - 04/11/22		Study Day 2 (obs window = ± 15 minutes around each time slot) - 04/12/22																	
Group	Animal ID	IM Challenge	SC Treatment	AM	PM	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0 mg/kg)	N	SS	SS	0	N	0	N	0	N	0	N	0	SS	0	SS	0	SS	0
	SA179G			N	N	N	0	N	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS	0
	BM842C			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	JR81CB			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	BC114A			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AL403H			SS	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)	N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AR787L			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	SL42B			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AK950J			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AK394MB			N	N	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	BM162L			N	N	N	0	N	0	N	0	SS	0	D	0	D	0	D	0	D	0

TCS = Total Clinical Severity Score

				Study Day 3 (obs window = ± 15 minutes around each time slot) - 04/13/22																					
Group	Animal ID	IM Challenge	SC Treatment	0000	TCS	0200	TCS	0400	TCS	0600	TCS	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0 mg/kg)	SS	0	SS	0	SS	0	SS	0	SS	0	N	0	N	0	N	0	N	0	N	0	P(1),H(1)	2	P(1),H(1)	2
	SA179G			SS	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS	0	SS,H(1)	1	SS,P(1),H(1)	2
	BM842C			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	H(1)	1	H(1)	1
	JR81CB			N	0	N	0	N	0	N	0	N	0	N	0	N	0	P(1)	1	P(1)	1	P(1)	1	P(1),H(1)	2	P(1),LB(1),H(1)	3
	BC114A			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	H(1)	1	H(1)	1
	AL403H			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	H(1)	1
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AR787L			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	SL42B			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AK950J			N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0
	AK394MB			N	0	N	0	N	0	N	0	N	0	NS	0	NS	0	H(1)	1	H(1)	1	H(1)	1	H(1)	1	H(1)	1
	BM162L			D	0	D	0	D	0	D	0	D	0	N	0	N	0	N	0	N	0	N	0	N	0	LB(1)	1

TCS = Total Clinical Severity Score

				Study Day 4 (obs window = ± 15 minutes around each time slot) - 04/14/22																					
Group	Animal ID	IM Challenge	SC Treatment	0000	TCS	0200	TCS	0400	TCS	0600	TCS	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0 mg/kg)	P(1),H(1)	2	P(1),H(1)	2	P(1),ND(1),LB(1), H(1)	4	P(1),ND(1),LB(1), H(1)	4	P(1),LB(1)	2	A,P(1),LB(1)	2	A,P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/IR(1),H(1)	3	A,P(1),L,LB(1),H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	P(1),L,OD(1),ND(1) LB/DR(1), H(1)	5
	SA179G			P(1),H(1)	2	P(1),H(1)	2	P(1),L,ND(1),H(1)	3	P(1),L,LB(1),H(1)	3	A,P(1),L,LB/DR(1), H(1)	3	A,P(1),L,OD(1), LB/DR(1),H(1)	4	A,P(1),L,OD(1),ND(1), LB/DR(1),H(1)	5	A,P(1),L,OD(1),ND(1), LB/DR/G(1),H(1)	5	NS,A,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5	NS,A,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5	NS,A,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5	NS,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5
	BM842C			P(1),H(1)	2	P(1),H(1)	2	P(1),L,LB(1),H(1)	3	P(1),L,LB(1),H(1)	3	P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P,(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(3), H(1)	5	P(1),L,LB/DR/G(3),H(1)	5	A,P(1),L,OD(1),LB/DR/G(1)),H(1)	4
	JR81CB			P(1),LB(1),H(1)	3	P(1),L,LB(1),H(1)	3	P(1),L,ND(1),LB(1),H(1)	4	P(1),L,LB(1),H(1)	3	A,P(1),L,LB(1),H(1)	3	A,P(1),L,LB(1),H(1)	3	A,P(1),L,LB/DR(1), H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3	A,P(1),L,LB/DR/G(1), H(1)	3
	BC114A			H(1)	1	L, H(1)	1	L, LB(1),H(1)	2	P(1),L,LB(1),H(1)	3	A,P(1),L,OD(1), LB/DR(1),H(1)	4	NS,A,P(1),L,OD(1), ND(1), LB/DR/G(1),H(1)	5	NS,A,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5	NS,A,P(1),L,OD(1), ND(1),LB/DR/G(1),H(1)	5	NS,A,P(1),L,LB/DR/G(1),H(1)	3	NS,P(1),L, LB/DR/G(1),PR(2)	4	P(1),SR/DR/G(1),PR(3)	5	FD(10)	10
	AL403H			LB(1),H(1)	2	LB(1),H(1)	2	L, LB(1),H(1)	2	L,LB(1),H(1)	2	A,P(1),L,LB(1),H(1)	3	A,P(1),L,LB(1),H(1)	3	A,P(1),L,LB/DR(1), H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	A,P(1),L,LB/DR(1),H(1)	3	P(1),L,OD(1),ND(1), LB/DR/G(1),H(1)	5	P(1),L,OD(1),ND(1), LB/DR/G(1),H(1)	5
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)	LB(1)	1	LB(1)	1	LB(1)	1	LB(1)	1	P(1),LB(1)	2	P(1),LB(1)	2	A,P(1),LB(1)	2	A,P(1),LB/DR(1),H(1)	3	A,P(1),LB/DR(1),H(1)	3	A,P(1),LB/DR(1),H(1)	3	A,P(1),LB/DR(1),H(1)	3	A,P(1),LB/DR/G(1), H(1)	3
	AR787L			N	0	N	0	LB(1),H(1)	2	LB(1),H(1)	2	A,LB(1),H(1)	2	A,LB(1),H(1)	2	A,LB(1),H(1)	2	A,LB/DR(1),H(1)	2	A,LB/DR(1),H(1)	2	A,LB/DR(1),H(1)	2	A,LB/DR(1),H(1)	2	A,P(1),LB/DR(1),H(1)	3
	SL42B			P(1)	1	P(1),IR	1	P(1),LB(1)	2	P(1),LB(1)	2	P(1),LB(1)	2	P(1),LB(1)	2	P(1),LB(1)	2	P(1),LB(1)	2	P(1),LB(1)	2	A,P(1),LB(1)	2	A,P(1),LB(1)	2	A,P(1),LB(1)	2
	AK950J			LB(1),H(1)	2	LB(1),H(1)	2	LB(1),H(1)	2	LB(1),H(1)	2	LB(1)	1	LB(1)	1	LB(1)	1	LB/DR(1)	1	A,LB(1)	1	A,LB(1)	1	A,LB(1)	1	A,LB(1)	1
	AK394MB			H(1)	1	H(1)	1	P(1),ND(1),LB(1), H(1)	4	P(1),ND(1),LB(1), H(1)	4	A,P(1),ND(1),LB(1), H(1)	4	A,P(1),ND(1),LB(1), H(1)	4	A,P(1),LB/DR(1),H(1)	3	A,P(1),LB/DR(1),H(1)	3	NS,A,P(1),L,LB/DR(1), H(1)	3	NS,A,P(1),L,LB/DR(1), H(1)	3	NS,A,P(1),L,LB/DR(1), H(1)	3	NS,A,P(1),L,LB/DR(1), H(1)	3
	BM162L			LB(1)	1	V,LB(1)	1	LB(1)	1	LB(1),H(1)	2	LB(1)	1	A	0	A	0	A	0	A,LB	0	A,LB,H(1)	1	A,LB,H(1)	1	A,LB/DR,H(1)	1

Prepared by: MV 6.13.22
QC Review by: JDV 6/13/22

Observation Key

N=Normal	A = ataxia	LB = labored respirations	LR=lateral recumbency
D= diarrhea	P = ptosis	SR = shallow respirations	PR = prostrate
NS = no stool	L = lethargic	DR = decreased respirations	FD = founddead
SS = Soft stool	OD = oral discharge	IR = increasedrespirations	
V = vomiting	ND = nasal discharge	FAR = forced abdominal respirations	
	H = hunchedposture	G = gasping	
( ) = clinical score		= Treatment Trigger	**TCS** =Total ClinicalSeverity Score

TCS = Total Clinical Severity Score

Study B06188 Clinical Observations

Clinical Scoring Table

ClinicalSign	Observation	Clinical Severity Score Assigned
Ptosis	Absent	0
	Slight	1
	Marked	2
Muscular Weakness	Normal	0
	Hunched Posture	1
	Prostrate -able to rise	2
	Prostrate -unable to rise	3
Respiratory Distress	Normal Breathing	0
	Open mouth shallow breathing/light gasping (thoracic)	1
	Strong Gasping and Jerking (forced abdominal breathing)	3
Oral Discharge	Absent	0
	Present	1
NasalDischarge	Absent	0
	Present	1
Lethality	Alive	0
	Dead	10

Study Day 5 (obs window = ± 15 minutes around each time slot) - 04/15/22

Group	Animal ID	IM Challenge	SC Treatment	0000	TCS	0200	TCS	0400	TCS	0600	TCS	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0mg/kg)	OD(1),DR,H(1)	2	P(1)OD(1), DR(1), H(1)	4	P(1),OD(1),ND(1), DR(1), H(1)	5	P(1), L, OD(1), ND(1), DR(1), H(1)	5	P(1), L, OD(1), ND(1), DR(1), H(1)	5	P(1), L, OD(1), ND(1),DR(1), H(1)	5	P(1), L, OD(1), ND(1), LB/DR(1), H(1)	5	P(1), L, OD(1), ND(1), DR/FAR(3), H(1)	7	P(1), L, OD(1), ND(1),DR/FAR(3), H(1)	7	P(1), L, OD(1), ND(1),DR/FAR(3), H(1)	7	P(1),L,OD(1), ND(1),FAR/G(3), H(1)	7	NS,P(1), L,OD(1),ND(1), FAR/G(3), PR(2)	8
	SA179G			L,LB(1), PR(3)	4	L,LB(1),LR(3)	4	L,SR(1), PR(3)	4	L,SR(1),PR(3), FD#0654 (10)	14																
	BM842C			P(1),LB(1),H(1)	3	P(1),LB(1),H(1)	3	P(1),LB(1),PR(2)	4	P(1),LB(1),PR(3)	5	P(1),LB/G(1),PR(3)	5	FD(10)	10												
	JR81CB			P(1),LB(1),H(1)	3	FD(10)	10																				
	BC114A																										
	AL403H			P(1),L,DR,LR(3)	4	P(1),L,SR(1),LR(3)	5	P(1),L,SR(1),PR(3)	5	P(1),L,SR(1),PR(3), FD#0629(10)	15																
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)	DR(1),H(1)	2	P(1),G(1),H(1)	3	P(1),G(1),H(1)	3	P(1),L,G(1),H(1), FD#0654(10)	13																
	AR787L			NS,H(1)	1	NS,LB,H(1)	1	NS,LB,H(1)	1	NS,L,LB,H(1)	1	NS,L,LB,H(1)	1	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,A,L,LB(1),H(1)	2		
	SL42B			P(1),LB,H(1)	2	P(1),LB,H(1)	2	P(1),LB,H(1)	2	L,LB,H(1)	1	L,ND(1),LB,H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,LB(1),H(1)	2	NS,L,ND(1),LB(1),H(1)	3	NS,L,LB(1),H(1)	2
	AK950J			LB,H(1)	1	LB,H(1)	1	SR,H(1)	1	L,SR,H(1)	1	L,SR,H(1)	1	NS,L,SR,H(1)	1	NS,L,SR,H(1)	1	NS,L,SR,H(1)	1	NS,L,DR/SR,H(1)	1	NS,L,DR/SR,H(1)	1	NS,L,DR/SR,H(1)	1	NS,L,DR/SR,H(1)	1
	AK394MB			NS, P(1),LB,H(1)	2	NS, P(1),LB,H(1)	2	NS, P(1),LB,H(1)	2	NS,L,LB,H(1)	1	NS, L,LB,H(1)	1	NS, L,LB,H(1)	1	NS, L,LB,H(1)	1	NS, L,LB,H(1)	1	NS, L,DR/LB,H(1)	1	NS, L,DR/LB,H(1)	1	NS, L,DR/LB,H(1)	1	NS, L,DR/LB,H(1)	1
	BM162L			LB,H(1)	1	LB,H(1)	1	LB,H(1)	1	LB,H(1)	1	LB,H(1)	1	NS,L,ND(1),LB,H(1)	2	NS,L,OD(1),LB,H(1)	2	NS,L,LB,H(1)	1	NS,L,LB,H(1)	1	NS,L,LB,H(1)	1	NS,L,OD(1),ND(1), LB/DR,H(1)	3	NS,L,OD(1),FAR(3),H(1)	5

TCS = Total Clinical Severity Score

Study Day 6 (obs window = ± 15 minutes around each time slot) - 04/16/22

Group	Animal ID	IM Challenge	SC Treatment	0000	TCS	0200	TCS	0400	TCS	0600	TCS	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0 mg/kg)	FD (10)	10																						
	SA179G																										
	BM842C																										
	JR81CB																										
	BC114A																										
	AL403H																										
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)																								
	AR787L			P(1),L,SR/DR(1), PR(2)	4	FD	10																				
	SL42B			P(1),L,DR(1),H(1)	3	FD	10																				
	AK950J			P(1),L,OD(1),SR(1), H(1)	4	P(1),L,OD(1), SR/G(1),H(1)	4	P(1),L,OD(1), SR/G(1),H(1)	4	P(1),L,SR/G(1),PR(3) FD#0626(10)	15																
	AK394MB			P(1),L,SR(1),H(1)	3	NS,P(1),L,SR(1), H(1)	3	NS,P(1),L, SR/DR(1), H(1)	3	NS,P(1),L,SR/DR(1), H(1)	3	NS,P(1),L,SR/DR(1), H(1)	3	NS,P(1),L,SR/DR(1), H(1)	3	NS,P(1),L,SR/DR(1), H(1)	3	NS,P(1),DR/SR(1),PR(3)FD #1428 (10)	15								
	BM162L			P(1),OD(1),SR(1), PR(2)	5	NS,P(1),L,OD(1), LB/SR(1),H(1)	4	NS,P(1),L,OD(1), LB/SR(1),H(1)	4	NS,P(1),L,OD(1), LB/SR(1),H(1)	4	NS,P(1),L,OD(1), LB/SR(1),H(1)	4	NS,P(1),L,OD(1), LB/SR(1),PR(2)	5	NS,P(1),L,OD(1), LB/SR(1),PR(2)	5	NS,P(1),L,OD(1), LB/SR(1),PR(2)	5	NS,P(1),L,OD(1), SR/DR/G(1),PR(2)	5	NS,P(1),L,OD(1), SR/DR/G(1),PR(2)	5	NS,P(1),L,OD(1), SR/DR/G(1),PR(2)	5	NS,P(1),L,OD(1), SR/DR(1),PR(2)	5

TCS = Total Clinical Severity Score

Study Day 7 (obs window = ± 15 minutes around each time slot) - 04/17/22

Group	Animal ID	IM Challenge	SC Treatment	0000	TCS	0200	TCS	0400	TCS	0600	TCS	0800	TCS	1000	TCS	1200	TCS	1400	TCS	1600	TCS	1800	TCS	2000	TCS	2200	TCS
1	BM813G	BoNT/A1	saline (0 mg/kg)																								
	SA179G																										
	BM842C																										
	JR81CB																										
	BC114A																										
	AL403H																										
2	GA669H	BoNT/A1	3.4-DAPP (4.12 mg/kg)																								
	AR787L																										
	SL42B																										
	AK950J																										
	AK394MB																										
	BM162L			FD(10)	10																						

TCS = Total Clinical Severity Score

Prepared by: MV 6.13.22
QC Review by: JDW 6/28/22

Appendix H Statistical Analysis Contributing Scientist Report on Survival Data

BATTELLE

Survival Efficacy Evaluation of 3,4-Diaminopyridine in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1

FINAL

Statistical Analysis Contributing Scientist Report
On Survival Data

January 10, 2023

Study Number B06188

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505 King Avenue
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Testing Facility
Battelle Biomedical Research Center (BBRC)
1425 State Route 142 (Plain City-Georgesville Road)
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SIGNATURES

Study Statistician:

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Contributing Statistician:

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Approved by:

[redacted]

1 INTRODUCTION

The purpose of this study was to evaluate the capacity of 3,4-diaminopyridine (3,4-DAP) following systemic intoxication with botulinum neurotoxin serotype A1 (BoNT/A1) to promote survival in the cynomolgus monkey model. This report summarizes the statistical analysis of survival data collected under Study B06188.

Twelve monkeys were randomized by body weight into one of two groups as shown in Table 1. Following BoNT/A1 administration via a single intramuscular injection, twice daily AM/PM observations were performed for the first 24 hours after challenge as manifestation of toxic signs was not anticipated during this timeframe. From ~24 hours after challenge until study termination at mortality or scheduled euthanasia, animals were clinically monitored at 2-hour intervals (± 15 minutes) for toxic signs of systemic botulism. Once an animal was triggered to receive subcutaneous treatments, repeat treatment frequency with 3,4-DAPP (or placebo) was performed every 2 hours (± 15 minutes) until mortality or scheduled euthanasia at 4 days (96 hours ± 30 minutes) after the initial onset of a clinical severity score of 1 or greater in any one of the selected toxic signs.

Table 1. Study Design.

Group	Challenge; Dose; Dose Route; Dose Volume	Treatment; Dose; Dose Route; Dose Volume	Endpoints	Number of Animals
1	BoNT/A1; 2.5x LD_{50}; IM; 0.0975 mL/kg	Saline; 0 mg/kg; SC; 2.06 mL/kg	Survival Clinical Observations	6
2		3,4-DAPP; 4.12 mg/kg; SC; 2.06 mL/kg		6

IM = intramuscular; SC = subcutaneous

2 STATISTICAL METHODS

The endpoint for survival was defined by the number of animals in each group that were alive or dead at 96 hours (± 30 minutes) after the onset of a clinical severity score of 1 or greater in any one of the selected toxic signs. Survival proportions with exact 95% confidence intervals were calculated for each group. A log-rank test was used to compare times to death after toxic sign onset (clinical severity of ≥ 1) between the two groups. A log-rank test was also used to compare times to death from challenge between the two groups. A Kaplan-Meier plot was estimated relative to the time of toxic sign onset (clinical severity of ≥ 1).

3 DEVIATIONS

There were no deviations considered to have an impact on the ability to interpret statistical analysis of survival data.

4 RESULTS

Table 2 displays the survival proportion and corresponding 95% confidence interval for each group. All animals died within 96 hours after toxic sign onset; therefore, comparison between the groups was not performed.

Table 3 summarizes the Kaplan-Meier median times to death from toxic sign onset in hours and corresponding 95% confidence intervals, standard deviations, as well as the p-value from the log-rank test comparing times to death relative to toxic sign onset between the groups. At the 0.05 level, time to death of the 3,4-DAPP treated group was significantly greater than that of the saline control group. Figure 1 presents the Kaplan-Meier curves for the time to death data relative to toxic sign onset.

Table 4 summarizes the Kaplan-Meier median times to death from challenge in hours and corresponding 95% confidence intervals, standard deviations, as well as the p-value from the log-rank test comparing times to death relative to challenge between the groups. At the 0.05 level, time to death of the 3,4-DAPP treated group was significantly greater than that of the saline control group.

5 CONCLUSIONS

All animals died, and times to death relative to toxic sign onset and relative to challenge of the 3,4-DAPP treated group were significantly longer than those of the saline control group.

Table 2. Survival Rates with Corresponding 95% Binomial Confidence Intervals.

Group	Treatment	No. Survived / No. in Group	Survival Proportion (95% Confidence Interval)
1	Saline	0 / 6	0.00 (0.00, 0.46)
2	3,4-DAPP	0 / 6	0.00 (0.00, 0.46)

Table 3. Kaplan-Meier Median Times to Death from Toxic Sign Onset with 95% Confidence Intervals and P-Value from Log-Rank Test Comparing Times to Death from Toxic Sign Onset.

Group	Treatment	No. Died / No. in Group	Kaplan-Meier Median Time to Death in Hours (95% Confidence Interval)	Standard Deviation	P-Value
1	Saline	6 / 6	35.38 (26.13, 52.13)	8.64	0.0416*
2	3,4-DAPP	6 / 6	52.20 (30.75, 73.90)	16.43	

* Times to death were significantly different between groups.

Table 4. Kaplan-Meier Median Times to Death from Challenge with 95% Confidence Intervals and P-Value from Log-Rank Test Comparing Times to Death from Challenge.

Group	Treatment	No. Died / No. in Group	Kaplan-Meier Median Time to Death in Hours (95% Confidence Interval)	Standard Deviation	P-Value
1	Saline	6 / 6	94.38 (85.23, 111.20)	8.94	0.0049*
2	3,4-DAPP	6 / 6	115.71 (94.30, 135.65)	13.95	

* Times to death were significantly different between groups.

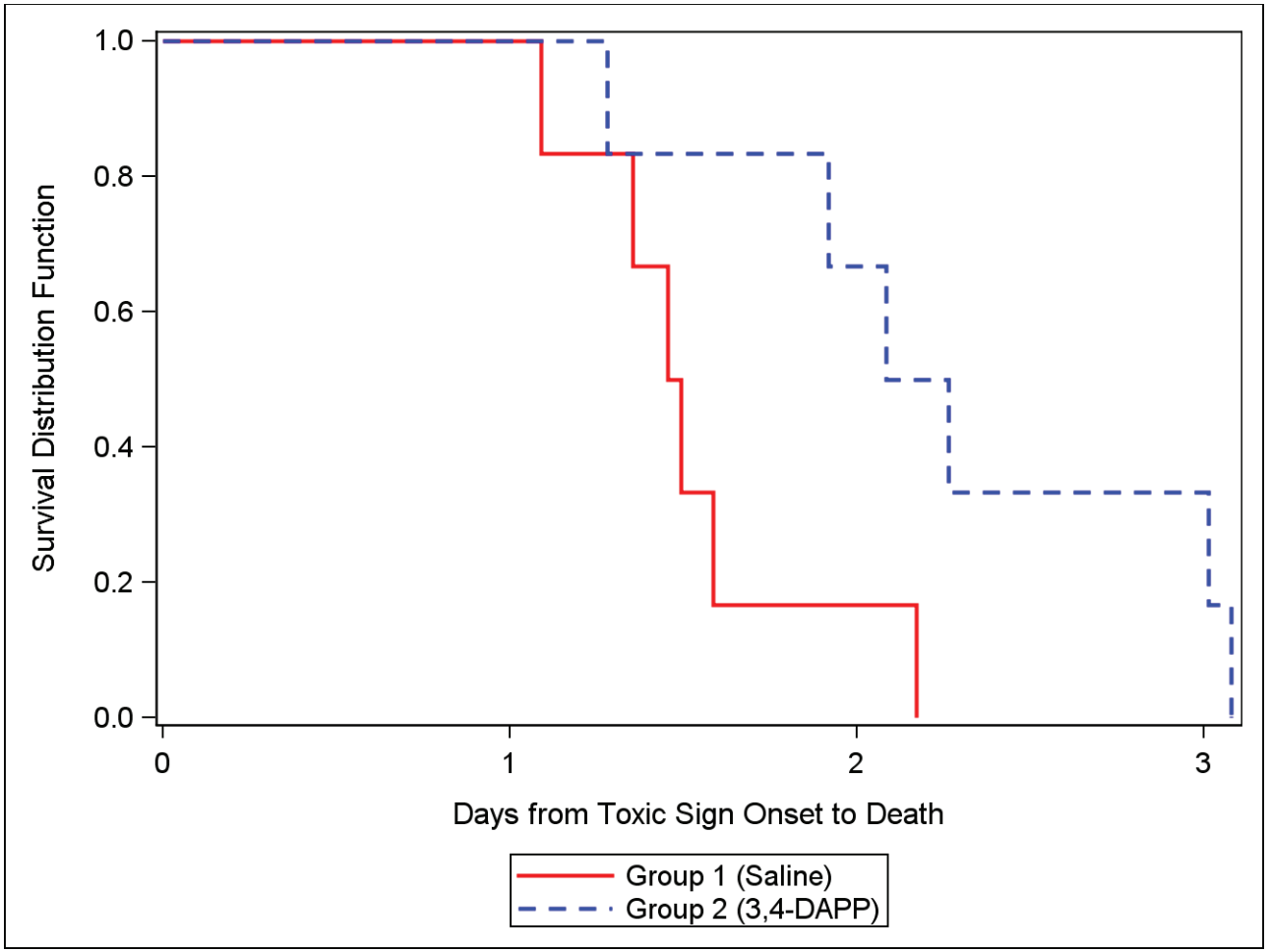


Figure 1. Kaplan-Meier Plot by Group.

Appendix I Statistical Analysis Contributing Scientist Report on Clinical Observation Data

BATTELLE

Survival Efficacy Evaluation of 3,4-Diaminopyridine in Cynomolgus Monkeys Intoxicated by Botulinum Neurotoxin Serotype A1

FINAL

Statistical Analysis Contributing Scientist Report
On Clinical Observations Data

January 10, 2023

Study Number B06188

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Columbus, OH 43201

Testing Facility
Battelle Biomedical Research Center (BBRC)
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SIGNATURES

Study Statistician:



Contributing Statistician:



Approved by:



1 INTRODUCTION

The purpose of this study was to evaluate the capacity of 3,4-diaminopyridine (3,4-DAP) following systemic intoxication with botulinum neurotoxin serotype A1 (BoNT/A1) to promote survival in the cynomolgus monkey model. This report summarizes the statistical analysis of clinical observations data collected under Study B06188.

Twelve monkeys were randomized by body weight into one of two groups as shown in Table 1. Following BoNT/A1 administration via a single intramuscular injection, twice daily AM/PM observations were performed for the first 24 hours after challenge as manifestation of toxic signs was not anticipated during this timeframe. From ~24 hours after challenge until study termination at mortality or scheduled euthanasia, animals were clinically monitored at 2-hour intervals (± 15 minutes) for toxic signs of systemic botulism. Once an animal was triggered to receive subcutaneous treatments, repeat treatment frequency with 3,4-DAPP (or placebo) was performed every 2 hours (± 15 minutes) until mortality or scheduled euthanasia at 4 days (96 hours ± 30 minutes) after the initial onset of a clinical severity score of 1 or greater in any one of the selected toxic signs.

Table 1. Study Design.

Group	Challenge; Dose; Dose Route; Dose Volume	Treatment; Dose; Dose Route; Dose Volume	Endpoints	Number of Animals
1	BoNT/A1; 2.5x LD_{50}; IM; 0.0975 mL/kg	Saline; 0 mg/kg; SC; 2.06 mL/kg	Survival Clinical Observations	6
2		3,4-DAPP; 4.12 mg/kg; SC; 2.06 mL/kg		6

IM = intramuscular; SC = subcutaneous

2 STATISTICAL METHODS

The proportions of observed clinical signs with exact 95% confidence intervals were calculated for each group at each time point. At each time point where at least three animals survived in both groups, a two-sided Boschloo's test was used to compare the proportions of each clinical observation between the two groups. At each time point, the Bonferroni-Holm multiple comparison procedure was used to control the overall Type I error rate of the tests across the different clinical observations at 5%. Across all time points, the numbers of animals that ever presented a clinical observation were calculated by group, and two-sided Boschloo's tests were used to compare between the two groups.

For total and respiratory severity scores, medians with interquartile ranges were calculated for each group at each time point. At each time point where at least three animals survived in both groups, a Kruskal-Wallis test was performed to determine if there were significant differences between the group medians. Medians and interquartile ranges were plotted for the severity scores.

3 DEVIATIONS

There were no deviations considered to have an impact on the ability to interpret statistical analysis of clinical observations data.

4 RESULTS

Table 2 displays the proportions of observed clinical signs and corresponding 95% confidence intervals for each group at each time point. Table 2 also contains p-values from Boschloo's tests comparing the proportions of each clinical observation between the two groups before and after the Bonferroni-Holm multiple comparison adjustments. Before the adjustments, at the 0.05 level, the proportion of animals that was normal was significantly greater in Group 2 than in Group 1 at 2000 and 2200 on Day 3; the proportion of animals that had hunched posture was significantly greater in Group 1 compared to Group 2 at 2000 and 2200 on Day 3 and at 0000, 0200, and 1200 on Day 4; the proportion of animals that had hunched posture was significantly greater in Group 2 compared to Group 1 from 0200 to 0600 on Day 5; the proportion of animals that was lethargic was significantly greater in Group 1 compared to Group 2 from 0400 to 2200 on Day 4; the proportion of animals that had decreased respirations was significantly greater in Group 1 compared to Group 2 at 1200 on Day 4; the proportion of animals that was gasping was significantly greater in Group 1 compared to Group 2 from 1600 to 2000 on Day 4; the proportion of animals that had ataxia was significantly greater in Group 2 compared to Group 1 at 2200 on Day 4; the proportion of animals that had oral discharge was significantly greater in Group 1 compared to Group 2 at 2200 on Day 4; and the proportion of animals that were prostrate was significantly greater in Group 1 compared to Group 2 at 0400 and 0600 on Day 5. After the adjustments, the proportion of animals that had hunched posture was significantly greater in Group 1 compared to Group 2 at 2200 on Day 3, and the proportion of animals that was lethargic was significantly greater in Group 1 compared to Group 2 at 0400, 0600, 1200, and 1400 on Day 4.

Table 3 displays the numbers of animals that ever presented a clinical observation across all time points by group, as well as p-values from Boschloo's tests comparing the proportions of each clinical observation between the two groups. The proportions were not significantly different between the two groups for any clinical observation.

Table 4 contains the medians and interquartile ranges of total severity scores by group at each time point, as well as p-values from Kruskal-Wallis tests comparing the median scores between the two groups. At the 0.05 level, the median scores of Group 1 were significantly greater than those of Group 2 from 2000 on Day 3 to 0200 on Day 4 and from 1000 on Day 4 to 0600 on Day 5. No comparison was performed after 0600 on Day 5 since less than three animals survived in Group 1. Figure 1 plots the median total severity scores and interquartile ranges where at least three animals survived by time point.

Table 5 contains the medians and interquartile ranges of respiratory severity scores by group at each time point, as well as p-values from Kruskal-Wallis tests comparing the median scores between the two groups. At the 0.05 level, the median scores of Group 1 were significantly greater than those of Group 2 from 0200 to 0600 on Day 5. No comparison was performed after 0600 on Day 5 since less than three animals survived in Group 1. Figure 2 plots the median respiratory severity scores and interquartile ranges where at least three animals survived by time point.

5 CONCLUSIONS

After adjusting for multiple comparisons, the proportion of animals with hunched posture was significantly greater in the saline control group than in the 3,4-DAPP treated group at 2200 on Day 3, and the proportions of animals that were lethargic were significantly greater in the saline control group than in the 3,4-DAPP treated group at 0400, 0600, 1200, and 1400 on Day 4. The median total severity scores of the saline control group were significantly greater than those of the 3,4-DAPP treated group at most time points from 2000 of Day 3 to 0600 of Day 5. The median respiratory severity scores of the saline control group were significantly greater than those of the 3,4-DAPP treated group from 0200 to 0600 on Day 5.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests.

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 1 AM	Normal	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 1 PM	Normal	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 2 1000	Normal	6/6 1.00 (0.54, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	--
Day 2 1200	Normal	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 2 1400	Normal	5/6 0.83 (0.36, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 2 1600	Normal	5/6 0.83 (0.36, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 2 1800	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 2 2000	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 2 2200	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 0000	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 0200	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 0400	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 0600	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 0800	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Diarrhea	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
Day 3 1000	Normal	5/6 0.83 (0.36, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	--
	No Stool	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 3 1200	Normal	5/6 0.83 (0.36, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	--
	No Stool	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 3 1400	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ptosis	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Hunched Posture	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 3 1600	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ptosis	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Hunched Posture	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 3 1800	Normal	4/6 0.67 (0.22, 0.96)	5/6 0.83 (0.36, 1.00)	1.0000	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ptosis	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Hunched Posture	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 3 2000	Normal	1/6 0.17 (0.00, 0.64)	5/6 0.83 (0.36, 1.00)	0.0386*	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ptosis	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.3940
	Hunched Posture	5/6 0.83 (0.36, 1.00)	1/6 0.17 (0.00, 0.64)	0.0386*	0.1157
Day 3 2200	Normal	0/6 0.00 (0.00, 0.46)	4/6 0.67 (0.22, 0.96)	0.0210*	--
	Soft Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ptosis	3/6 0.50 (0.12, 0.88)	0/6 0.00 (0.00, 0.46)	0.0676	0.2027
	Hunched Posture	6/6 1.00 (0.54, 1.00)	1/6 0.17 (0.00, 0.64)	0.0063*	0.0254*
	Labored Respirations	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 4 0000	Normal	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	--
	Ptosis	4/6 0.67 (0.22, 0.96)	1/6 0.17 (0.00, 0.64)	0.1460	0.2920
	Hunched Posture	6/6 1.00 (0.54, 1.00)	2/6 0.33 (0.04, 0.78)	0.0210*	0.0630
	Labored Respirations	2/6 0.33 (0.04, 0.78)	3/6 0.50 (0.12, 0.88)	1.0000	1.0000
Day 4 0200	Normal	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	--
	Vomiting	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Ptosis	4/6 0.67 (0.22, 0.96)	1/6 0.17 (0.00, 0.64)	0.1460	0.7300
	Lethargic	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.7881
	Hunched Posture	6/6 1.00 (0.54, 1.00)	2/6 0.33 (0.04, 0.78)	0.0210*	0.1260
	Labored Respirations	2/6 0.33 (0.04, 0.78)	3/6 0.50 (0.12, 0.88)	1.0000	1.0000
	Increased Respirations	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
Day 4 0400	Ptosis	4/6 0.67 (0.22, 0.96)	2/6 0.33 (0.04, 0.78)	0.3877	0.9087
	Lethargic	5/6 0.83 (0.36, 1.00)	0/6 0.00 (0.00, 0.46)	0.0063*	0.0317*
	Nasal Discharge	3/6 0.50 (0.12, 0.88)	1/6 0.17 (0.00, 0.64)	0.3029	0.9087
	Hunched Posture	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.2703
	Labored Respirations	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 4 0600	Ptosis	5/6 0.83 (0.36, 1.00)	2/6 0.33 (0.04, 0.78)	0.1460	0.5840
	Lethargic	5/6 0.83 (0.36, 1.00)	0/6 0.00 (0.00, 0.46)	0.0063*	0.0317*
	Nasal Discharge	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Hunched Posture	6/6 1.00 (0.54, 1.00)	4/6 0.67 (0.22, 0.96)	0.1970	0.5911
	Labored Respirations	6/6 1.00 (0.54, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
Day 4 0800	Ataxia	4/6 0.67 (0.22, 0.96)	2/6 0.33 (0.04, 0.78)	0.3877	1.0000
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.4730
	Lethargic	5/6 0.83 (0.36, 1.00)	0/6 0.00 (0.00, 0.46)	0.0063*	0.0508
	Oral Discharge	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Nasal Discharge	0/6 0.00 (0.00, 0.46)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Hunched Posture	5/6 0.83 (0.36, 1.00)	2/6 0.33 (0.04, 0.78)	0.1460	0.7300
	Labored Respirations	6/6 1.00 (0.54, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Decreased Respirations	3/6 0.50 (0.12, 0.88)	0/6 0.00 (0.00, 0.46)	0.0676	0.4730
Day 4 1000	No Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ataxia	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Lethargic	5/6 0.83 (0.36, 1.00)	0/6 0.00 (0.00, 0.46)	0.0063*	0.0635
	Oral Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.9851
	Nasal Discharge	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Hunched Posture	5/6 0.83 (0.36, 1.00)	2/6 0.33 (0.04, 0.78)	0.1460	0.8760
	Labored Respirations	6/6 1.00 (0.54, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	1.0000
	Decreased Respirations	3/6 0.50 (0.12, 0.88)	0/6 0.00 (0.00, 0.46)	0.0676	0.6082
	Gasping	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 4 1200	No Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ataxia	6/6 1.00 (0.54, 1.00)	4/6 0.67 (0.22, 0.96)	0.1970	1.0000
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.4730
	Lethargic	6/6 1.00 (0.54, 1.00)	0/6 0.00 (0.00, 0.46)	0.0005*	0.0049*
	Oral Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	1.0000
	Nasal Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	1.0000
	Hunched Posture	6/6 1.00 (0.54, 1.00)	2/6 0.33 (0.04, 0.78)	0.0210*	0.1681
	Labored Respirations	6/6 1.00 (0.54, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	1.0000
	Decreased Respirations	6/6 1.00 (0.54, 1.00)	1/6 0.17 (0.00, 0.64)	0.0063*	0.0571
	Gasping	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 4 1400	No Stool	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Ataxia	6/6 1.00 (0.54, 1.00)	4/6 0.67 (0.22, 0.96)	0.1970	0.9851
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Lethargic	6/6 1.00 (0.54, 1.00)	0/6 0.00 (0.00, 0.46)	0.0005*	0.0049*
	Oral Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.9851
	Nasal Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	0.9851
	Hunched Posture	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Labored Respirations	6/6 1.00 (0.54, 1.00)	5/6 0.83 (0.36, 1.00)	1.0000	1.0000
	Decreased Respirations	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Gasping	3/6 0.50 (0.12, 0.88)	0/6 0.00 (0.00, 0.46)	0.0676	0.6082
Day 4 1600	No Stool	2/6 0.33 (0.04, 0.78)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Ataxia	6/6 1.00 (0.54, 1.00)	4/6 0.67 (0.22, 0.96)	0.1970	1.0000
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Lethargic	6/6 1.00 (0.54, 1.00)	1/6 0.17 (0.00, 0.64)	0.0063*	0.0698
	Oral Discharge	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Nasal Discharge	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Hunched Posture	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Labored Respirations	6/6 1.00 (0.54, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Decreased Respirations	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Increased Respirations	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Gasping	4/6 0.67 (0.22, 0.96)	0/6 0.00 (0.00, 0.46)	0.0210*	0.2101
Day 4 1800	No Stool	2/6 0.33 (0.04, 0.78)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Ataxia	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6082
	Lethargic	6/6 1.00 (0.54, 1.00)	1/6 0.17 (0.00, 0.64)	0.0063*	0.0698
	Oral Discharge	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Nasal Discharge	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Hunched Posture	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Labored Respirations	6/6 1.00 (0.54, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Decreased Respirations	5/6 0.83 (0.36, 1.00)	3/6 0.50 (0.12, 0.88)	0.3029	1.0000
	Gasping	4/6 0.67 (0.22, 0.96)	0/6 0.00 (0.00, 0.46)	0.0210*	0.2101
	Prostrate	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 4 2000	No Stool	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Ataxia	3/6 0.50 (0.12, 0.88)	6/6 1.00 (0.54, 1.00)	0.0676	0.6758
	Ptosis	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6758
	Lethargic	5/6 0.83 (0.36, 1.00)	1/6 0.17 (0.00, 0.64)	0.0386*	0.4243
	Oral Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	1.0000
	Nasal Discharge	2/6 0.33 (0.04, 0.78)	0/6 0.00 (0.00, 0.46)	0.1970	1.0000
	Hunched Posture	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Labored Respirations	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Shallow Respirations	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
	Decreased Respirations	6/6 1.00 (0.54, 1.00)	3/6 0.50 (0.12, 0.88)	0.0676	0.6758
	Gasping	5/6 0.83 (0.36, 1.00)	0/6 0.00 (0.00, 0.46)	0.0063*	0.0762
	Prostrate	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 4 2200	No Stool	1/6 0.17 (0.00, 0.64)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Ataxia	2/6 0.33 (0.04, 0.78)	6/6 1.00 (0.54, 1.00)	0.0210*	0.2311
	Ptosis	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Lethargic	5/6 0.83 (0.36, 1.00)	1/6 0.17 (0.00, 0.64)	0.0386*	0.3472
	Oral Discharge	4/6 0.67 (0.22, 0.96)	0/6 0.00 (0.00, 0.46)	0.0210*	0.2311
	Nasal Discharge	3/6 0.50 (0.12, 0.88)	0/6 0.00 (0.00, 0.46)	0.0676	0.5406
	Hunched Posture	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Labored Respirations	5/6 0.83 (0.36, 1.00)	6/6 1.00 (0.54, 1.00)	1.0000	1.0000
	Decreased Respirations	5/6 0.83 (0.36, 1.00)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Gasping	4/6 0.67 (0.22, 0.96)	1/6 0.17 (0.00, 0.64)	0.1460	1.0000
	Found Dead	1/6 0.17 (0.00, 0.64)	0/6 0.00 (0.00, 0.46)	1.0000	1.0000
Day 5 0000	No Stool	0/5 0.00 (0.00, 0.52)	2/6 0.33 (0.04, 0.78)	0.3247	1.0000
	Ptosis	3/5 0.60 (0.15, 0.95)	2/6 0.33 (0.04, 0.78)	0.5488	1.0000
	Lethargic	2/5 0.40 (0.05, 0.85)	0/6 0.00 (0.00, 0.46)	0.1143	1.0000
	Oral Discharge	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
	Hunched Posture	3/5 0.60 (0.15, 0.95)	6/6 1.00 (0.54, 1.00)	0.1143	1.0000
	Labored Respirations	3/5 0.60 (0.15, 0.95)	4/6 0.67 (0.22, 0.96)	1.0000	1.0000
	Decreased Respirations	2/5 0.40 (0.05, 0.85)	1/6 0.17 (0.00, 0.64)	0.4536	1.0000
	Lateral Recumbency	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
	Prostrate	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 5 0200	No Stool	0/5 0.00 (0.00, 0.52)	2/6 0.33 (0.04, 0.78)	0.3247	1.0000
	Ptosis	3/5 0.60 (0.15, 0.95)	3/6 0.50 (0.12, 0.88)	1.0000	1.0000
	Lethargic	2/5 0.40 (0.05, 0.85)	0/6 0.00 (0.00, 0.46)	0.1143	1.0000
	Oral Discharge	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
	Hunched Posture	2/5 0.40 (0.05, 0.85)	6/6 1.00 (0.54, 1.00)	0.0363*	0.3998
	Labored Respirations	2/5 0.40 (0.05, 0.85)	5/6 0.83 (0.36, 1.00)	0.2266	1.0000
	Shallow Respirations	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
	Decreased Respirations	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
	Gasping	0/5 0.00 (0.00, 0.52)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Lateral Recumbency	2/5 0.40 (0.05, 0.85)	0/6 0.00 (0.00, 0.46)	0.1143	1.0000
	Found Dead	1/5 0.20 (0.01, 0.72)	0/6 0.00 (0.00, 0.46)	0.3247	1.0000
Day 5 0400	No Stool	0/4 0.00 (0.00, 0.60)	2/6 0.33 (0.04, 0.78)	0.3091	1.0000
	Ptosis	3/4 0.75 (0.19, 0.99)	3/6 0.50 (0.12, 0.88)	0.5293	1.0000
	Lethargic	2/4 0.50 (0.07, 0.93)	0/6 0.00 (0.00, 0.46)	0.0638	0.5743
	Oral Discharge	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Nasal Discharge	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Hunched Posture	1/4 0.25 (0.01, 0.81)	6/6 1.00 (0.54, 1.00)	0.0106*	0.1164
	Labored Respirations	1/4 0.25 (0.01, 0.81)	4/6 0.67 (0.22, 0.96)	0.3962	1.0000
	Shallow Respirations	2/4 0.50 (0.07, 0.93)	1/6 0.17 (0.00, 0.64)	0.3709	1.0000
	Decreased Respirations	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Gasping	0/4 0.00 (0.00, 0.60)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Prostrate	3/4 0.75 (0.19, 0.99)	0/6 0.00 (0.00, 0.46)	0.0106*	0.1164
Day 5 0600	No Stool	0/4 0.00 (0.00, 0.60)	2/6 0.33 (0.04, 0.78)	0.3091	1.0000
	Ptosis	3/4 0.75 (0.19, 0.99)	1/6 0.17 (0.00, 0.64)	0.1094	1.0000
	Lethargic	3/4 0.75 (0.19, 0.99)	5/6 0.83 (0.36, 1.00)	1.0000	1.0000
	Oral Discharge	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Nasal Discharge	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Hunched Posture	1/4 0.25 (0.01, 0.81)	6/6 1.00 (0.54, 1.00)	0.0106*	0.1270
	Labored Respirations	1/4 0.25 (0.01, 0.81)	4/6 0.67 (0.22, 0.96)	0.3962	1.0000
	Shallow Respirations	2/4 0.50 (0.07, 0.93)	1/6 0.17 (0.00, 0.64)	0.3709	1.0000
	Decreased Respirations	1/4 0.25 (0.01, 0.81)	0/6 0.00 (0.00, 0.46)	0.2105	1.0000
	Gasping	0/4 0.00 (0.00, 0.60)	1/6 0.17 (0.00, 0.64)	1.0000	1.0000
	Prostrate	3/4 0.75 (0.19, 0.99)	0/6 0.00 (0.00, 0.46)	0.0106*	0.1270
	Found Dead	2/4 0.50 (0.07, 0.93)	1/6 0.17 (0.00, 0.64)	0.3709	1.0000

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 5 0800	No Stool	0/2 0.00 (0.00, 0.84)	2/5 0.40 (0.05, 0.85)	--	--
	Ptosis	2/2 1.00 (0.16, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/2 0.50 (0.01, 0.99)	4/5 0.80 (0.28, 0.99)	--	--
	Oral Discharge	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Nasal Discharge	1/2 0.50 (0.01, 0.99)	1/5 0.20 (0.01, 0.72)	--	--
	Hunched Posture	1/2 0.50 (0.01, 0.99)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	1/2 0.50 (0.01, 0.99)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/2 0.00 (0.00, 0.84)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Gasping	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Prostrate	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
Day 5 1000	No Stool	0/2 0.00 (0.00, 0.84)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/2 0.50 (0.01, 0.99)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Nasal Discharge	1/2 0.50 (0.01, 0.99)	1/5 0.20 (0.01, 0.72)	--	--
	Hunched Posture	1/2 0.50 (0.01, 0.99)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/2 0.00 (0.00, 0.84)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/2 0.00 (0.00, 0.84)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
	Found Dead	1/2 0.50 (0.01, 0.99)	0/5 0.00 (0.00, 0.52)	--	--
Day 5 1200	No Stool	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	1/5 0.20 (0.01, 0.72)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Hunched Posture	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	1/1 1.00 (0.03, 1.00)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
Day 5 1400	No Stool	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Hunched Posture	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Forced Abdominal Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

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Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 5 1600	No Stool	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Hunched Posture	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/1 1.00 (0.03, 1.00)	2/5 0.40 (0.05, 0.85)	--	--
	Forced Abdominal Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
Day 5 1800	No Stool	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Hunched Posture	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	1/1 1.00 (0.03, 1.00)	2/5 0.40 (0.05, 0.85)	--	--
	Forced Abdominal Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
Day 5 2000	No Stool	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	1/5 0.20 (0.01, 0.72)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	2/5 0.40 (0.05, 0.85)	--	--
	Hunched Posture	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	0/1 0.00 (0.00, 0.98)	3/5 0.60 (0.15, 0.95)	--	--
	Forced Abdominal Respirations	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Gasping	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 5 2200	No Stool	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Ataxia	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Ptosis	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Lethargic	1/1 1.00 (0.03, 1.00)	5/5 1.00 (0.48, 1.00)	--	--
	Oral Discharge	1/1 1.00 (0.03, 1.00)	1/5 0.20 (0.01, 0.72)	--	--
	Nasal Discharge	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Hunched Posture	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Labored Respirations	0/1 0.00 (0.00, 0.98)	3/5 0.60 (0.15, 0.95)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	1/5 0.20 (0.01, 0.72)	--	--
	Decreased Respirations	0/1 0.00 (0.00, 0.98)	2/5 0.40 (0.05, 0.85)	--	--
	Forced Abdominal Respirations	1/1 1.00 (0.03, 1.00)	1/5 0.20 (0.01, 0.72)	--	--
	Gasping	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
	Prostrate	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
Day 6 0000	Ptosis	0/1 0.00 (0.00, 0.98)	5/5 1.00 (0.48, 1.00)	--	--
	Lethargic	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Oral Discharge	0/1 0.00 (0.00, 0.98)	2/5 0.40 (0.05, 0.85)	--	--
	Hunched Posture	0/1 0.00 (0.00, 0.98)	3/5 0.60 (0.15, 0.95)	--	--
	Shallow Respirations	0/1 0.00 (0.00, 0.98)	4/5 0.80 (0.28, 0.99)	--	--
	Decreased Respirations	0/1 0.00 (0.00, 0.98)	2/5 0.40 (0.05, 0.85)	--	--
	Prostrate	0/1 0.00 (0.00, 0.98)	2/5 0.40 (0.05, 0.85)	--	--
	Found Dead	1/1 1.00 (0.03, 1.00)	0/5 0.00 (0.00, 0.52)	--	--
Day 6 0200	No Stool	--	2/5 0.40 (0.05, 0.85)	--	--
	Ptosis	--	3/5 0.60 (0.15, 0.95)	--	--
	Lethargic	--	3/5 0.60 (0.15, 0.95)	--	--
	Oral Discharge	--	2/5 0.40 (0.05, 0.85)	--	--
	Hunched Posture	--	3/5 0.60 (0.15, 0.95)	--	--
	Labored Respirations	--	1/5 0.20 (0.01, 0.72)	--	--
	Shallow Respirations	--	3/5 0.60 (0.15, 0.95)	--	--
	Gasping	--	1/5 0.20 (0.01, 0.72)	--	--
	Found Dead	--	2/5 0.40 (0.05, 0.85)	--	--
Day 6 0400	No Stool	--	2/3 0.67 (0.09, 0.99)	--	--
	Ptosis	--	3/3 1.00 (0.29, 1.00)	--	--
	Lethargic	--	3/3 1.00 (0.29, 1.00)	--	--
	Oral Discharge	--	2/3 0.67 (0.09, 0.99)	--	--
	Hunched Posture	--	3/3 1.00 (0.29, 1.00)	--	--
	Labored Respirations	--	1/3 0.33 (0.01, 0.91)	--	--
	Shallow Respirations	--	3/3 1.00 (0.29, 1.00)	--	--
	Decreased Respirations	--	1/3 0.33 (0.01, 0.91)	--	--
	Gasping	--	1/3 0.33 (0.01, 0.91)	--	--

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

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Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 6 0600	No Stool	--	2/3 0.67 (0.09, 0.99)	--	--
	Ptosis	--	3/3 1.00 (0.29, 1.00)	--	--
	Lethargic	--	3/3 1.00 (0.29, 1.00)	--	--
	Oral Discharge	--	1/3 0.33 (0.01, 0.91)	--	--
	Hunched Posture	--	2/3 0.67 (0.09, 0.99)	--	--
	Labored Respirations	--	1/3 0.33 (0.01, 0.91)	--	--
	Shallow Respirations	--	3/3 1.00 (0.29, 1.00)	--	--
	Decreased Respirations	--	1/3 0.33 (0.01, 0.91)	--	--
	Gasping	--	1/3 0.33 (0.01, 0.91)	--	--
	Prostrate	--	1/3 0.33 (0.01, 0.91)	--	--
	Found Dead	--	1/3 0.33 (0.01, 0.91)	--	--
Day 6 0800	No Stool	--	2/2 1.00 (0.16, 1.00)	--	--
	Ptosis	--	2/2 1.00 (0.16, 1.00)	--	--
	Lethargic	--	2/2 1.00 (0.16, 1.00)	--	--
	Oral Discharge	--	1/2 0.50 (0.01, 0.99)	--	--
	Hunched Posture	--	2/2 1.00 (0.16, 1.00)	--	--
	Labored Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Shallow Respirations	--	2/2 1.00 (0.16, 1.00)	--	--
	Decreased Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
Day 6 1000	No Stool	--	2/2 1.00 (0.16, 1.00)	--	--
	Ptosis	--	2/2 1.00 (0.16, 1.00)	--	--
	Lethargic	--	2/2 1.00 (0.16, 1.00)	--	--
	Oral Discharge	--	1/2 0.50 (0.01, 0.99)	--	--
	Hunched Posture	--	1/2 0.50 (0.01, 0.99)	--	--
	Labored Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Shallow Respirations	--	2/2 1.00 (0.16, 1.00)	--	--
	Decreased Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Prostrate	--	1/2 0.50 (0.01, 0.99)	--	--
Day 6 1200	No Stool	--	2/2 1.00 (0.16, 1.00)	--	--
	Ptosis	--	2/2 1.00 (0.16, 1.00)	--	--
	Lethargic	--	2/2 1.00 (0.16, 1.00)	--	--
	Oral Discharge	--	1/2 0.50 (0.01, 0.99)	--	--
	Hunched Posture	--	1/2 0.50 (0.01, 0.99)	--	--
	Labored Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Shallow Respirations	--	2/2 1.00 (0.16, 1.00)	--	--
	Decreased Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Prostrate	--	1/2 0.50 (0.01, 0.99)	--	--

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

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Table 2. Proportions of Clinical Observations with Corresponding 95% Binomial Confidence Intervals by Time Point and P-Values from Boschloo's Tests (Continued).

Study Day and Time	Observation	Proportion Positive (95% Confidence Interval)		Unadjusted P-Value#	Adjusted P-Value#
		Group 1 (Saline)	Group 2 (3,4-DAPP)		
Day 6 1400	No Stool	--	2/2 1.00 (0.16, 1.00)	--	--
	Ptosis	--	2/2 1.00 (0.16, 1.00)	--	--
	Lethargic	--	1/2 0.50 (0.01, 0.99)	--	--
	Oral Discharge	--	1/2 0.50 (0.01, 0.99)	--	--
	Labored Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Shallow Respirations	--	2/2 1.00 (0.16, 1.00)	--	--
	Decreased Respirations	--	1/2 0.50 (0.01, 0.99)	--	--
	Prostrate	--	2/2 1.00 (0.16, 1.00)	--	--
	Found Dead	--	1/2 0.50 (0.01, 0.99)	--	--
Day 6 1600	No Stool	--	1/1 1.00 (0.03, 1.00)	--	--
	Ptosis	--	1/1 1.00 (0.03, 1.00)	--	--
	Lethargic	--	1/1 1.00 (0.03, 1.00)	--	--
	Oral Discharge	--	1/1 1.00 (0.03, 1.00)	--	--
	Shallow Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Decreased Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Gasping	--	1/1 1.00 (0.03, 1.00)	--	--
	Prostrate	--	1/1 1.00 (0.03, 1.00)	--	--
Day 6 1800	No Stool	--	1/1 1.00 (0.03, 1.00)	--	--
	Ptosis	--	1/1 1.00 (0.03, 1.00)	--	--
	Lethargic	--	1/1 1.00 (0.03, 1.00)	--	--
	Oral Discharge	--	1/1 1.00 (0.03, 1.00)	--	--
	Shallow Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Decreased Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Gasping	--	1/1 1.00 (0.03, 1.00)	--	--
	Prostrate	--	1/1 1.00 (0.03, 1.00)	--	--
Day 6 2000	No Stool	--	1/1 1.00 (0.03, 1.00)	--	--
	Ptosis	--	1/1 1.00 (0.03, 1.00)	--	--
	Lethargic	--	1/1 1.00 (0.03, 1.00)	--	--
	Oral Discharge	--	1/1 1.00 (0.03, 1.00)	--	--
	Shallow Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Decreased Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Gasping	--	1/1 1.00 (0.03, 1.00)	--	--
	Prostrate	--	1/1 1.00 (0.03, 1.00)	--	--
Day 6 2200	No Stool	--	1/1 1.00 (0.03, 1.00)	--	--
	Ptosis	--	1/1 1.00 (0.03, 1.00)	--	--
	Lethargic	--	1/1 1.00 (0.03, 1.00)	--	--
	Oral Discharge	--	1/1 1.00 (0.03, 1.00)	--	--
	Shallow Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Decreased Respirations	--	1/1 1.00 (0.03, 1.00)	--	--
	Prostrate	--	1/1 1.00 (0.03, 1.00)	--	--
Day 7 0000	Found Dead	--	1/1 1.00 (0.03, 1.00)	--	--

Unadjusted p-value is for two-sided Boschloo's test comparing proportions of the groups. For adjusted p-values, overall error rate at each time point was controlled at 5% using the Bonferroni-Holm multiple comparison procedure.

* Proportions were significantly different between the groups.

-- No observation was available, no comparison was performed due to having less than three animals surviving in both groups, or result for observations of "Normal" was not included when adjusting for multiple comparisons.

Table 3. Number of Animals that Ever Presented a Clinical Observation Across All Time Points and P-Values from Boschloo's Tests.

Observation	Number of Animals that Ever Presented a Clinical Observation (N=6)		Boschloo's P-Value[#]
	Group 1 (Saline)	Group 2 (3,4-DAPP)	
Normal	6	6	1.0000
Diarrhea	0	1	1.0000
No Stool	3	5	0.3029
Soft Stool	3	1	0.3029
Vomiting	0	1	1.0000
Ataxia	6	6	1.0000
Ptosis	6	6	1.0000
Lethargic	6	6	1.0000
Oral Discharge	5	2	0.1460
Nasal Discharge	5	3	0.3029
Hunched Posture	6	6	1.0000
Labored Respirations	6	6	1.0000
Shallow Respirations	3	4	1.0000
Decreased Respirations	6	6	1.0000
Increased Respirations	1	1	1.0000
Forced Abdominal Respirations	1	1	1.0000
Gasping	6	3	0.0676
Lateral Recumbency	2	0	0.1970
Prostrate	5	4	1.0000
Found Dead	6	6	1.0000

P-value is for two-sided Boschloo's test comparing proportions of the groups. Proportions were not significantly different between the groups for any clinical observation.

Table 4. Descriptive Statistics for Total Severity Scores by Time Point and P-Values from Kruskal-Wallis Tests.

Study Day and Time	Group 1 (Saline)		Group 2 (3,4-DAPP)		Kruskal-Wallis P-Value#
	N	Median (Interquartile Range)	N	Median (Interquartile Range)	
Day 1	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 2	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1200	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1400	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1600	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1800	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 2000	6	1.0 (1.0)	6	0.0 (0.0)	0.0231*
Day 3 2200	6	1.5 (1.0)	6	0.0 (1.0)	0.0110*
Day 4 0000	6	2.0 (0.0)	6	1.0 (0.0)	0.0248*
Day 4 0200	6	2.0 (0.0)	6	1.0 (0.0)	0.0248*
Day 4 0400	6	3.0 (2.0)	6	2.0 (1.0)	0.0931
Day 4 0600	6	3.0 (0.0)	6	2.0 (0.0)	0.0750
Day 4 0800	6	3.0 (0.0)	6	2.0 (1.0)	0.0668
Day 4 1000	6	3.0 (1.0)	6	2.0 (1.0)	0.0484*
Day 4 1200	6	3.0 (2.0)	6	2.0 (1.0)	0.0073*
Day 4 1400	6	3.0 (2.0)	6	2.0 (2.0)	0.0163*
Day 4 1600	6	3.0 (0.0)	6	2.0 (2.0)	0.0200*
Day 4 1800	6	3.5 (2.0)	6	2.0 (2.0)	0.0123*
Day 4 2000	6	5.0 (2.0)	6	2.0 (2.0)	0.0077*
Day 4 2200	5	5.0 (1.0)	6	2.5 (2.0)	0.0107*
Day 5 0000	5	3.0 (1.0)	6	1.5 (1.0)	0.0105*
Day 5 0200	4	4.0 (1.0)	6	1.5 (1.0)	0.0122*
Day 5 0400	4	4.5 (1.0)	6	1.5 (1.0)	0.0089*
Day 5 0600	4	5.0 (0.5)	6	1.0 (0.0)	0.0056*
Day 5 0800	2	5.0 (--)	5	1.0 (0.0)	--
Day 5 1000	1	5.0 (--)	5	2.0 (1.0)	--
Day 5 1200	1	5.0 (--)	5	2.0 (1.0)	--
Day 5 1400	1	7.0 (--)	5	1.0 (1.0)	--
Day 5 1600	1	7.0 (--)	5	1.0 (1.0)	--
Day 5 1800	1	7.0 (--)	5	1.0 (1.0)	--
Day 5 2000	1	7.0 (--)	5	2.0 (2.0)	--
Day 5 2200	1	8.0 (--)	5	2.0 (1.0)	--
Day 6 0000	0	--	5	4.0 (1.0)	--
Day 6 0200	0	--	3	4.0 (1.0)	--
Day 6 0400	0	--	3	4.0 (1.0)	--
Day 6 0600	0	--	3	4.0 (2.0)	--
Day 6 0800	0	--	2	3.5 (--)	--
Day 6 1000	0	--	2	4.0 (--)	--
Day 6 1200	0	--	2	4.0 (--)	--
Day 6 1400	0	--	2	5.0 (--)	--
Day 6 1600	0	--	1	5.0 (--)	--
Day 6 1800	0	--	1	5.0 (--)	--
Day 6 2000	0	--	1	5.0 (--)	--
Day 6 2200	0	--	1	5.0 (--)	--

P-value is for Kruskal-Wallis test comparing medians of the groups.
* Medians were significantly different between the groups.
-- Median and/or interquartile range could not be evaluated due to having no or less than three observations available, or no comparison was performed due to having less than three animals surviving in both groups.

Table 5. Descriptive Statistics for Respiratory Severity Scores by Time Point and P-Values from Kruskal-Wallis Tests.

Study Day and Time	Group 1 (Saline)		Group 2 (3,4-DAPP)		Kruskal-Wallis P-Value#
	N	Median (Interquartile Range)	N	Median (Interquartile Range)	
Day 1	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 2	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1200	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1400	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1600	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 1800	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 2000	6	0.0 (0.0)	6	0.0 (0.0)	1.0000
Day 3 2200	6	0.0 (0.0)	6	0.0 (0.0)	0.3173
Day 4 0000	6	0.0 (1.0)	6	0.0 (1.0)	1.0000
Day 4 0200	6	0.0 (1.0)	6	0.0 (1.0)	1.0000
Day 4 0400	6	1.0 (0.0)	6	1.0 (0.0)	1.0000
Day 4 0600	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 0800	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 1000	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 1200	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 1400	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 1600	6	1.0 (0.0)	6	1.0 (0.0)	0.3173
Day 4 1800	6	1.0 (0.0)	6	1.0 (0.0)	0.1757
Day 4 2000	6	1.0 (0.0)	6	1.0 (0.0)	0.1757
Day 4 2200	5	1.0 (0.0)	6	1.0 (0.0)	0.3613
Day 5 0000	5	1.0 (1.0)	6	0.0 (0.0)	0.1561
Day 5 0200	4	1.0 (0.0)	6	0.0 (0.0)	0.0143*
Day 5 0400	4	1.0 (0.0)	6	0.0 (0.0)	0.0143*
Day 5 0600	4	1.0 (0.0)	6	0.0 (0.0)	0.0143*
Day 5 0800	2	1.0 (--)	5	0.0 (0.0)	--
Day 5 1000	1	1.0 (--)	5	0.0 (1.0)	--
Day 5 1200	1	1.0 (--)	5	0.0 (1.0)	--
Day 5 1400	1	3.0 (--)	5	0.0 (1.0)	--
Day 5 1600	1	3.0 (--)	5	0.0 (1.0)	--
Day 5 1800	1	3.0 (--)	5	0.0 (1.0)	--
Day 5 2000	1	3.0 (--)	5	0.0 (1.0)	--
Day 5 2200	1	3.0 (--)	5	1.0 (1.0)	--
Day 6 0000	0	--	5	1.0 (0.0)	--
Day 6 0200	0	--	3	1.0 (0.0)	--
Day 6 0400	0	--	3	1.0 (0.0)	--
Day 6 0600	0	--	3	1.0 (0.0)	--
Day 6 0800	0	--	2	1.0 (--)	--
Day 6 1000	0	--	2	1.0 (--)	--
Day 6 1200	0	--	2	1.0 (--)	--
Day 6 1400	0	--	2	1.0 (--)	--
Day 6 1600	0	--	1	1.0 (--)	--
Day 6 1800	0	--	1	1.0 (--)	--
Day 6 2000	0	--	1	1.0 (--)	--
Day 6 2200	0	--	1	1.0 (--)	--

P-value is for Kruskal-Wallis test comparing medians of the groups.
* Medians were significantly different between the groups.
-- Median and/or interquartile range could not be evaluated due to having no or less than three observations available, or no comparison was performed due to having less than three animals surviving in both groups.

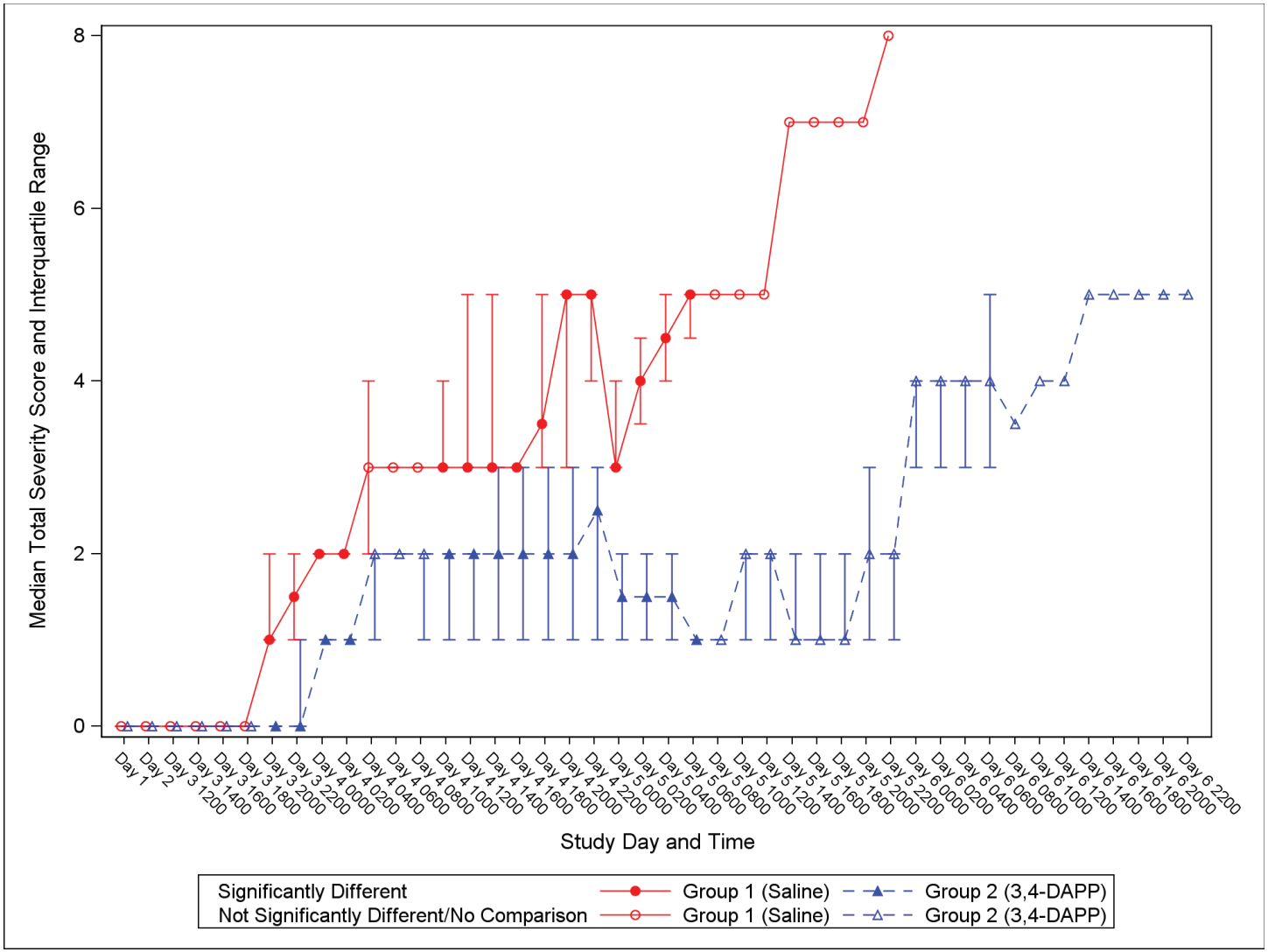


Figure 1. Median Total Severity Scores and Interquartile Ranges by Time Point.

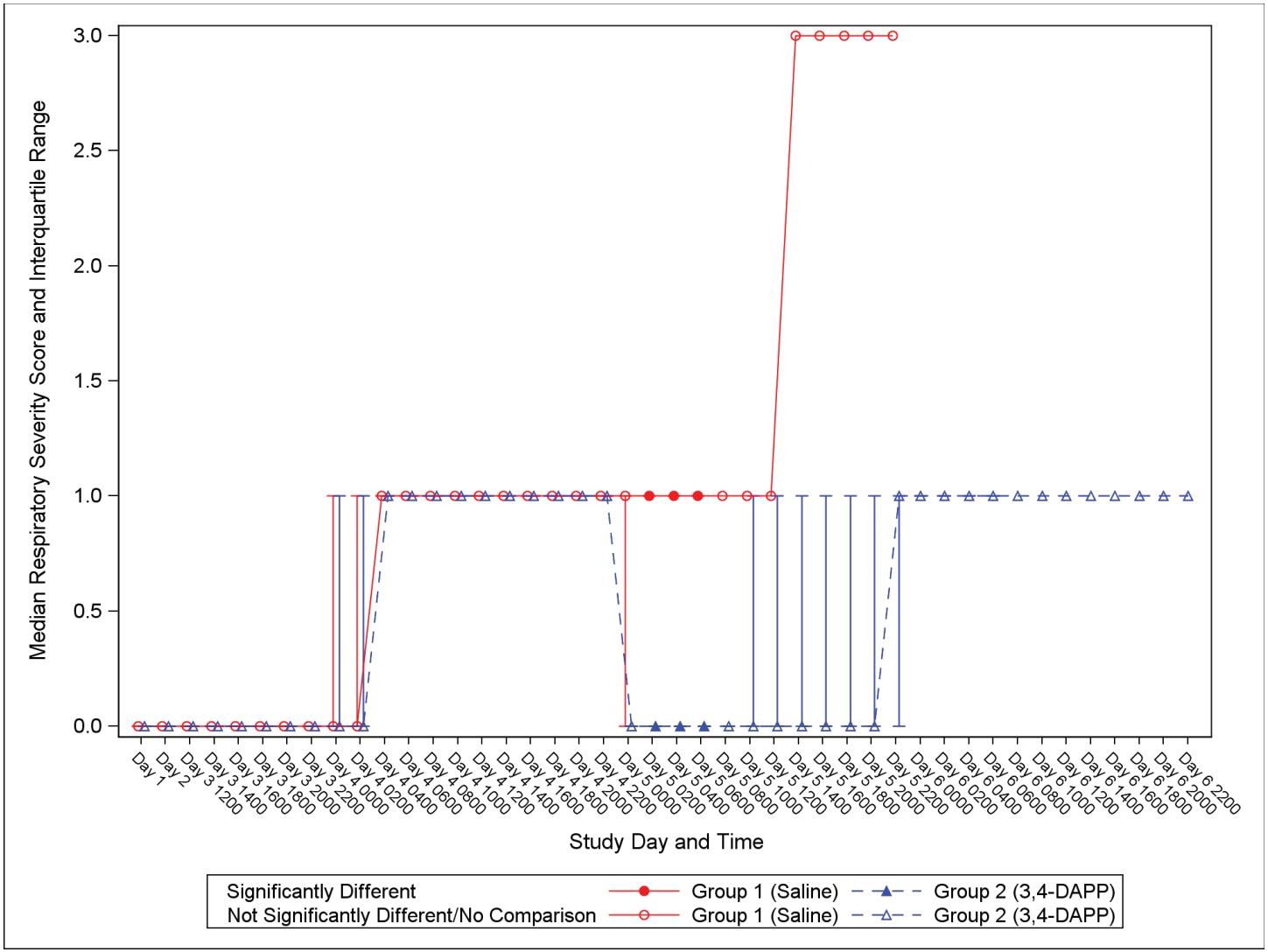


Figure 2. Median Respiratory Severity Scores and Interquartile Ranges by Time Point.