Current Ver. 27January2026

Date: March 27, 2026

Title: **Evaluation of ETSP # 613009 in the Mouse Maximal Electroshock Seizure (MES) and Rotarod Motor Impairment Assays**

Purpose: To determine the effect of **intraperitoneal (i.p.)** drug administration of an investigational compound on acute seizures in the mouse MES model and motor function in the rotarod assay.

Study ID: 1

Dose (mg/kg): 0 ☑ This is a vehicle test

Strain / Sex / Supplier: CF - 1 / Male / Charles River

Vehicle: 6.25% DMSO, 93.75% (50% Captisol in water)

Compound Supply Batch: NA

Laboratory: Experiments were performed by the Epilepsy Therapy Screening Program Contract Site at the University of Utah under contract number 75N95022C00007

Experimenter: 19

Notebook Reference: 551: 113-150

INTRODUCTION: The investigational compound was evaluated by the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP) in a rodent preclinical seizure model. The compound was screened for its ability to prevent the incidence of evoked seizure in the mouse maximal electroshock seizure (MES) model. In addition, an assessment of motor impairment in the rotarod assay and general behavioral observations for the compound was performed prior to the MES test. The route of administration for these tests was i.p.

RESULTS:

Maximal Electroshock Seizure (MES) Model

Prevention of seizures by an investigational compound in the MES model is indicative of the compound's ability to prevent the spread of seizure activity in a model of generalized tonic-clonic seizures (1) Performance of the investigational compound against MES-induced tonic extension seizures in male mice is shown in Table 1.

Rotarod Assay

The rotarod test is used to identify potential adverse effects of the investigational compound on the motor function of mice. Assessment of impairment in rotarod is performed following drug administration and immediately before seizure tests. Performance of the mice in the rotarod assay is shown in Table 1.

Current Ver. 27January2026

Table 1: Profile of Antiseizure Activity and Rotarod Performance of ETSP # 613009 in Male Mice (i.p. administration)

Time (hr.):			0.5			2					
Test	**Form.**	**Dose (mg/kg)**	**N/F**	**C[a]**	**# affected**	**N/F**	**C[a]**	**# affected**	**N/F**	**C[a]**	**# affected**
MES	Sol.	0	0/4	4	2	0/4					
Rotarod	Sol.	0	0/8			0/8					
Survival at 72 hr. post-drug admin.											

The data for each condition is presented as N/F, where N = number of animals protected (MES) or displaying adverse motor effects (rotarod) and F = number of animals tested. Any adverse effects, including deaths, are noted. For 72-hour survival assessment, N = animals that survived and F = number of animals tested. C = comment code. An observer blinded to treatment confirms behavioral observations. Form. = Formulation. Sol. = Solution. Sus. = Suspension

[a] Comment code description is provided in Table 2.

Table 2: Adverse Effects Observed in Animals Dosed with ETSP # 613009 (i.p. administration)

Test	Time (hr.)	Dose (mg/kg)	Comment Code	Description	Count
MES	0.5	0	4	Death following tonic extension	2
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	

Current Ver. 27January2026

DEVIATIONS FROM STANDARD PROTOCOL:	ADDITIONAL COMMENTS:
None	These are the results for the vehicle group only

Current Ver. 27January2026

APPENDIX A
METHODS

Methods are attached as Appendix A. An overview of the screening flow and the description of models used by the ETSP can be found at https://panache.ninds.nih.gov/

The *in vivo* data in this report is reported as per the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines)

Animals

Male albino CF - 1 mice (18 - 30 g; Charles River, Kingston, NY) were used as experimental animals. All animals were housed, fed, and handled in a manner consistent with the recommendations in the National Research Council publication, "Guide for the Care and Use of Laboratory Animals" (2). Mice were group housed in a pathogen-free facility under a 12-h light/12-h dark light cycle and had access to food (Teklad Global 2920X) and water ad libitum, except during experiments. No insecticides capable of altering hepatic drug metabolism enzymes were used in the animal facilities. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals. All protocols involving the use of animals have been approved by the Institutional Animal Care and Use Committee at the University of Utah.

Testing Paradigm

Animals were transferred to the experimental room approximately 30 minutes to one hour before testing, for an acclimation period, coded and randomized using GraphPad (http://graphpad.com/quickcalcs/randMenu). N = 4 mice/dose/time point were used in the test. Animals were tested at two time point(s) and - dose(s) given by i.p. administration in a volume of 0.01 ml/g body weight in mice. Additional doses and time points can be used if supported by known pharmacology. The results for each condition are presented as N/F, where N = number of animals protected and F = number of animals tested. For tests of impairment (rotarod), N = number of animals displaying adverse effects and F = number of animals tested. Any adverse effects, including deaths, were noted. The experimenter was blinded to the identity of experimental drug and an observer blinded to treatment confirmed behavioral observations.

Maximal Electroshock Seizure (MES) Test

A drop of 0.5% tetracaine hydrochloride in 0.9% saline (anesthetic/electrolyte) solution was applied to the eyes of each animal prior to placement of the corneal electrodes and a 50 mA, 60 Hz, 0.2 seconds electrical stimulus was delivered via corneal electrodes by an apparatus similar to that originally described by Woodbury and Davenport (3). Mice not displaying hindlimb tonic extension were considered protected (4-6).

Rotarod Assay

Shortly before MES test the performance of mice in rotarod test was examined to identify potential effect of the test compound on motor functions (7). When a mouse is placed on a 1-inch knurled rod that rotates at a speed of 6 r.p.m., the animal can maintain its equilibrium for long periods of time. The mouse was considered impaired if

it fell off this rotating rod three times during a 1-minute period.

72-hour Survival Assessment

Vehicle group animals were not assessed for 72-hour survival

Exclusion of animals and data points:

No animals were excluded from this study

Biospecimen Collection:

No blood or tissue collection

Vehicle Testing Details:

No associated ESTP# for the vehicle

Compound Preparation for Administration

The test substance was prepared according to ETSP instruction in
6.25% DMSO, 93.75% (50% Captisol in water)
The compound was administered i.p. in a volume of 0.01 ml/g body weight in mice.

Formulation Images

Current Ver. 27January2026

Comment Codes

Code #	Description
2	Died during test without seizure
3	Death following continuous seizure
4	Death following tonic extension
5	Death following clonic seizure
11	Anesthesia (total loss of feeling or sensation, unresponsive to tail pinch and tapping of the eye)
12	Ataxia (lack of voluntary coordination of muscle movements, can include wobbly gait)
13	Loss of righting reflex (unable to turn over when placed in a dorsal recumbent position)
14	Unable to grasp rotarod (inability to hold on to rotarod in order to begin test)
15	Minimal motor impairment
16	Loss of muscle tone (soft, with low muscle tone)
19	Sedated (very calm or appear to be sleeping, but will respond to external stimuli)
22	Continuous seizure activity
23	Clonic seizures (muscle convulsions of the forelimbs and/or hindlimbs)
24	Intense, repeated jumping straight up
25	Myoclonic jerks (non-rhythmic muscle twitch, jerk, shake or spasm)
26	Wild running (frantic running)
27	Myoclonic jerks (non-rhythmic muscle twitch, jerk, shake or spasm) following investigational compound administration but before the induction of a seizure test
28	Writhing (a stretch, tension to one side, extension of hind legs, contraction of the abdomen, or twisting of the trunk)
29	Hyperactivity (increased velocity of movement, faster motion than typical)
31	Severe tremors (strong rhythmic muscle contraction, shaking movements in the limbs or body leading to complete or near incapacitation)
32	Exophthalmos (eye bulging)
33	Tremors (rhythmic muscle contraction, shaking movements in the limbs or body)
34	Muscle spasms (continuous or recurrent muscle contraction or rigidity)
35	Wet dog shakes (a brief, ~1 second shaking of entire body. not restricted to single body part)
36	Stretching and rolling (extension/elongation of the body; rolling onto one side with or without completely exposing the ventral body surface)
37	Retropulsion (backward locomotion or backward circling)
38	Arching (arching of the back)
41	Diarrhea (loose, watery stool)
42	Salivation (noticeable saliva outside the mouth)
45	Piloerection (hairs become erect and bristle due to hair follicle contraction i.e., goose bumps)
49	Hyperesthesia (increase in sensitivity for all senses, i.e., jumping at noises, running or jumping when touched)
55	Vocalizations (noises audible to humans)
56	Excessive grooming (intense, excessive or disproportionate body cleaning; may be restricted to specific body parts, with or without visible signs of tissue damage)
61	Urinary staining (pigmented urine)
62	Bloody urine (bright red urine)
80	Cold tail (tail feels cold when touched)
81	Cold to the touch (animal’s body feels colder than typical - more severe than cold tail, above)
Z	Other - described in table

Current Ver. 27January2026

Table A1. Antiseizure Activity and Minimal Toxicity of Prototype Anti-Seizure Medications (ASMs) in Maximal Electroshock (MES) Model in Male Mice

ASM	i.p. Administration		p.o. Administration	
	TD_{50}[a](mg/kg) Rotarod	ED_{50}[a] (mg/kg) and PI[b] MES	TD_{50}[a](mg/kg) Rotarod	ED_{50}[a] (mg/kg) and PI[b] MES
Carbamazepine	40.6 (33.2 - 43.7)	7.3 (6.4 - 10.6) **PI 5.5**	125.5 (74.1 - 168.8)	11.6 (8.4 - 13.6) **PI 10.8**
Clobazam	16.8 (11.5 - 23.7)	19.7 (13.4 - 28.3) **PI 0.9**	196.7 (118.6 - 333.0)	29.88 (17.3 - 43.3) **PI 6.6**
Clonazepam	2.4 (1.9 - 3.4)	20.1 (7.2 - 40.9) **PI 0.1**	2.0 (1.4 – 2.9)	42.8 (25.8 – 64.8) **PI 0.1**
Ethosuximide	361.4 (326.9-399.5)	> 370 **PI < 1**	554.6 (345.2 - 1122.1)	> 800 **PI < 1**
Ezogabine	59.1 (35.5-105.4)	23.2 (18.8-32.3) **PI 2.5**	> 500	57 (38.9 - 75.8) **PI 8.8**
Felbamate	453.0 (363.2 - 563.2)	49.3 (39.8 - 78.3) **PI 9.2**	309.8 (220.1 - 416.6)	57.5 (49.2 - 63.7) **PI 5.4**
Gabapentin	> 500	77.2 (56.5 - 98.7) **PI > 6.5**	NT	NT
Lacosamide	23.8 (21.0-28.4)	3.4 (2-4.2) **PI 7**	NT	NT
Lamotrigine	90.1 (63.9 – 112.1)	5.4 (4.8 – 6.1) **PI 16.7**	135.4 (116.9 - 154.6)	4.4 (3.7 - 5.7) **PI 30.1**
Levetiracetam	> 500	> 500	> 500	> 500
Phenytoin	41.6 (32.6-48.9)	4.4 (3.1-5.4) **PI 9.4**	127.1 (79.8 - 164.5)	10.1 (7.8-12.0) **PI 12.6**
Tiagabine	7.6 (4.4-10.3)	>8 **PI < 1**	33.2 (25.4 - 46.8)	NT
Topiramate	233.5 (183.2-309.6)	18.3 (12.7-22.0) **PI 12.8**	NT	NT
Valproate	568.9 (539.4-604.9)	255 (227.6-278.2) **PI 2.2**	> 600	NT

[a] 95% confidence interval ( ).
[b] Protective Index; PI = TD_{50}/ED_{50}.
NT = not tested, ND = not determined
A minimum of 4 doses with 8 animals per dose were tested to determine values.

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