Final Report

28-Day Oral Study in Rats
(ToxDocs Study Number 11-340, MTDID 8391)

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Experiment In-Life Start Date: 9-1-2011

Experiment In-Life End Date: 10-12-2011

Study Summary

The objective of this study (ToxDocs Study Number 11-340) was to investigate the bilateral pupillary reflex responses in male Sprague Dawley rats (n=12/dose group) during 28 daily oral doses with either vehicle control (DI water) or MTDID 8391 (ammonium perfluorobutyrate, NH_4^+PFBA, 30 and 150 mg/kg/day).

All rats survived until scheduled necropsy. Twenty-four hours after the last treatment, ocular tissues (n=12/dose group) were removed and processed accordingly for Western blot and retina whole-mount evaluation performed in the laboratory of Dr. Don Fox at University of Houston, School of Optometry. No other tissue samples were collected. Western blots and histology analysis for the ocular samples are pending at the time of preparation of this report and will be appended to this report when they become available.

Study Objective

The objective of this study was to investigate the bilateral pupillary reflex responses in male Sprague Dawley rats during a 28-day oral dose study with either vehicle control (DI water) or MTDID 8391 (ammonium perfluorobutyrate). Rats received 28 consecutive daily doses of either vehicle control or MTDID 8391 (NH_4^+PFBA, 150 mg/kd/day) via oral gavage. Periodic bilateral pupillary reflex were evaluated during the treatment period. Twenty-four hours after the last treatment, ocular tissues from all rats were removed and processed accordingly for Western blot and retina whole-mount histology evaluation by Dr. Don Fox, University of Houston, School of Optometry.

Experimental Procedures & Observation

Test Material:

Identity	MTDID 8391
Other Identifiers	Ammonium perfluorobutyrate, E-19895, NH_4^+PFBA
Lot/Batch Number	3M NB# 140499-10/11
Composition	CF_3-CF_2-CF_2-$CO_2^-NH_4^+$
Molecular Weight	231
Purity/Strength	98.8% pure, 28.9% in water
Specific Gravity	NA
Appearance	Liquid
Stability	Stable

Species Selection:

The Animal Usage Application protocol (AUA 2011-0001) requires the use of a small rodent species to optimize use of test material. Sprague Dawley rats were selected as they are commonly used in oral toxicity testing to generate data for human health risk assessment.

Animal Husbandry:

Housing – Rats from the main study group were individually housed in standard solid-bottom cages except during dosing which requiring their removal from cages.

Diet/Water – commercially available certified rodent chow and tap water were provided to all animals *ad libitum* throughout the study.

Environment – Environmental controls for the animal room were set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour, and a 12-hour light/dark cycle. The 12-hour light/dark cycle was altered on the day of pupillary reflex examination, in which the room was kept dark in the morning until the pupillary reflex examination was completed.

Exposure:

Dose Group	N	Daily Dose umol/kg (mg/kg)[a]	Dose Volume (mL/kg)	MTDID 8391 Concentration	Scheduled Euthanasia
Control (distilled water)	12	0	5	0	24 hours post last dose
150 mg/kg/day MTDID 8391	12	650 (150)	5	30 mg/mL	24 hours post last dose

[a] Dose refers to the salt form of the main component of the test article.

MTDID 8391 was prepared in vehicle (distilled water) as 30 mg/mL. Five mL of the dosing solution was orally given to rats per kg body weights for 28 consecutive days.

Observation:

Each animal was observed daily for morbidity/mortality.

Body Weights:

All rats were weighed weekly. The body weight information was used to calculate the daily dosing volumes for the subsequent 7 days until next scheduled weighing. All rats were also weighed prior to necropsy.

Pupillary Reflex Evaluation (PRE):

Pre-dosing and interim pupillary reflex evaluations were performed on all rats throughout the study based on methods provided by Dr. Don Fox.

Necropsy and Specimen Collection:

All the rats were euthanized by decapitation 24 hours after last dose. Immediately after decapitation, ocular tissues were removed and processed accordingly for either Western bolts or retina whole-mount histology.

Results & Discussion

All rats survived until scheduled necropsy. Data for body weights are summarized in Table 1. All MTDID 8391-dosed rats gain weight similar to control rats. Bilateral pupillary reflex evaluation results are presented in Table 2. The corresponding ocular Western blots and histology data are pending, and they will be appended to this report when they become available.

Signatures

Report Prepared By:

Jill Hart 27 MAR 2012

Jill Hart
Study Director

Date

Reviewed By:

26 MAR 2012

John Butenhoff, Ph.D., DABT, CIH
Principle Investigator

Date

Table 1: Data for body weights and liver weights

Dose Group	Daily Dose (mg/kg)	Animal #	Tail Mark	Pre-Dose Body Weight (g)	Week 2 Body Weight (g)	Week 3 Body Weight (g)	Week 4 Body Weight (g)	Pre-Necropsy Body Weight (g)
1	0	1R307	I	285	308	335	342	352
		1R308	II	269	296	320	341	355
		1R309	III	255	281	307	323	343
		1R310	IV	256	278	296	314	329
		1R311	V	283	308	337	359	385
		1R312	VI	258	280	302	322	339
2	0	1R313	VII	267	295	325	342	362
		1R314	VIII	268	298	326	345	356
		1R315	IX	268	296	323	340	359
3	0	1R316	X	268	292	318	343	363
		1R317	XI	282	313	341	363	378
		1R318	XII	264	287	311	332	351
4	150	1R319	I	254	287	306	319	334
		1R320	II	267	302	328	342	357
		1R321	III	285	322	343	356	371
		1R322	IV	266	294	315	326	344
		1R323	V	264	295	309	318	333
		1R324	VI	273	311	335	352	375
5	150	1R325	VII	280	311	336	363	374
		1R326	VIII	289	319	353	379	389
		1R327	IX	265	301	326	348	368
6	150	1R328	X	281	309	333	345	350
		1R329	XI	284	310	328	337	351
		1R330	XII	289	316	337	353	371

Table 2: Bilateral pupillary reflex evaluation (PRE) data

				1st pre-dose PRE, 9-1-2011		2nd pre-dose PRE, 9-8-2011		1st PRE, 9-15-2011		2nd PRE, 9-22-2011		3rd PRE, 9-29-2011	
Dose Group	Daily Dose (mg/kg)	Animal Number	Tail Mark	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
1	0	1R307	I	+	+	+	+	+	+	+	+	+	+
		1R308	II	+	+	+	+	+	+	+	+	+	+
		1R309	III	+	+	+	+	+	+	+	+	+	+
		1R310	IV	+	+	+	+	+	+	+	+	+	+
		1R311	V	+	+	+	+	+	+	+	+	+	+
		1R312	VI	+	+	+	+	+	+	+	+	+	+
2	0	1R313	VII	+	+	+	+	+	+	+	+	+	+
		1R314	VIII	+	+	+	+	+	+	+	+	+	+
		1R315	IX	+	+	+	+	+	+	+	+	+	+
3	0	1R316	X	+	+	+	+	+	+	+	+	+	+
		1R317	XI	+	+	+	+	+	+	+	+	+	+
		1R318	XII	+	+	+	+	+	+	+	+	+	+
4	150	1R319	I	+	+	+	+	+	+	-	-	-	-
		1R320	II	+	+	+	+	+	+	-	-	-	+
		1R321	III	+	+	+	+	+	+	-	-	-	-
		1R322	IV	+	+	+	+	+	+	-	-	-	+
		1R323	V	+	+	+	+	+	+	-	-	+	-
		1R324	VI	+	+	+	+	+	+	-	-	-	-
5	150	1R325	VII	+	+	+	+	+	+	-	-	-	+
		1R326	VIII	+	+	+	+	+	+	-	-	-	-
		1R327	IX	+	+	+	+	+	+	-	-	-	-
6	150	1R328	X	+	+	+	+	+	+	+	-	-	-
		1R329	XI	+	+	+	+	+	+	-	-	-	-
		1R330	XII	+	+	+	+	+	+	-	-	-	-

"+" = Pupils responded to light stimuli and constricted normally

"-" = Pupils responded to light stimuli but with slower constriction