Final Report

28-Day Oral Study in Rats
(ToxDocs Study Number 10-152, MTDID 8391)

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Experiment In-Life Start Date: 5-18-2010

Experiment In-Life End Date: 7-1-2010

Study Summary

The objective of this study (ToxDocs Study Number 10-152) was to investigate the bilateral pupillary reflex responses in male Sprague Dawley rats (n=18/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). Interim serum samples were also collected weekly from a subset of rats (n=6/dose group) during the study.

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 histology and Western blot evaluation performed in the laboratory of Dr. Don Fox at University of Houston, School of Optometry. In addition, retina, serum, liver, and brain samples were harvested (n=6/dose group) and analyzed for PFBA concentrations by LC/MS-MS.

Twenty-four hours after the last oral treatment, mean (± SD) serum and liver PFBA concentrations were 14.25 ± 10.08 ug/mL and 4.46 ± 4.20 ug/g, respectively, for the 30 mg/kg/day dose group rats, and serum and liver PFBA concentrations were 25.08 ± 11.02 ug/mL and 11.20 ± 7.37 ug/g, respectively, for the 150 mg/kg/day dose group rats. MTDID 8391 concentrations were less than 1 ug/g in each of the retina and brain s collected (cerebellum, cortex, and rest of the brain).

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, 30 or 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 histology and biochemical analysis in accordance with methods provided by Dr. Don Fox, University of Houston, School of Optometry. In addition, to characterize the toxicokinetic profile of MTDID 8391 during the 28-day treatment period, interim blood samples were collected from a subgroup of rats via tail vein bleeding on a weekly basis. Twenty-four hours after the last treatment, ocular and brain tissues were removed and stored frozen pending LC-MS/MS analyses.

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 2008-0459) 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)	18	0	5	0	24 hours post last dose
30 mg/kg/day MTDID 8391	18	130 (30)	5	6 mg/mL	24 hours post last dose
150 mg/kg/day MTDID 8391	18	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 6 and 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 when practical 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-dosing and interim pupillary reflex evaluations were performed on all rats throughout the study based on methods provided by Dr. Don Fox.

Interim Specimen Collection:

Interim (weekly) blood samples were collected from a subset of rats (n=6/dose group) via tail vein bleeding. The blood samples were allowed to clot for a period of 15 to 30 minutes at room temperature and spun down in a centrifuge at 2000 x g for 15 minutes to obtain serum. The serum samples were carefully transferred to labeled 0.6-mL polypropylene microcentrifuge tubes. Serum, urine, and fecal samples were stored frozen at -70°C pending LC-MS/MS analysis.

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, histology, or LC-MS/MS evaluation. Liver and brain tissues (sectioned and labeled as: cerebellum, cortex, and the rest of the brain) were also removed from a subset of rats pending LC-MS/MS analyses.

Results & Discussion

All rats survived until scheduled necropsy. Data for body weights and liver 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.

LC-MS/MS data are presented in Table 3. Twenty-four hours after the last oral treatment, serum and liver PFBA concentrations were 14.25 ± 10.08 ug/mL and 4.46 ± 4.20 ug/g, respectively, for

the 30 mg/kg/day dose group rats, and serum and liver PFBA concentrations were 25.08 ± 11.02 ug/mL and 11.20 ± 7.37 ug/g, respectively, for the 150 mg/kg/day dose group rats. PFBA concentrations were less than 1 ug/g in each of the retina and brain regions collected (cerebellum, cortex, and rest of the brain).

Signatures

Report Prepared By:

Jill Hart 21 JAN 2011

Jill Hart
Study Director

Date

Reviewed By:

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John Butenhoff, Ph.D., DABT, CIH
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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)	Liver Weight (g)
1	0	0R292	I	242	281	311	337	360	NA
		0R293	II	234	274	301	324	345	NA
		0R294	III	239	284	320	345	379	NA
		0R295	IV	233	270	296	312	335	NA
		0R296	V	244	286	324	250	377	NA
		0R297	VI	241	285	311	326	347	NA
2	0	OR298	VII	261	297	332	358	376	NA
		0R299	VIII	241	278	309	331	355	NA
		0R300	IX	232	272	302	325	342	NA
		0R301	X	245	285	321	348	371	NA
		0R302	XI	244	277	304	321	343	NA
		0R303	XII	254	287	315	339	359	NA
3	30	0R304	I	249	299	334	351	368	16.7
		0R305	II	229	269	302	319	338	13.7
		0R306	III	241	285	317	336	364	16.0
		0R307	IV	220	256	276	286	301	14.3
		0R308	V	227	267	297	318	336	15.3
		0R309	VI	234	273	304	319	333	15.6
4	30	0R310	VII	250	275	277	314	343	NA
		0R311	VIII	213	243	255	265	280	NA
		0R312	IX	272	314	351	375	396	NA
		0R313	X	244	279	311	333	351	NA
		0R314	XI	237	271	291	312	329	NA
		0R315	XII	250	295	325	351	368	NA
5	150	0R316	I	242	286	314	330	347	NA
		0R317	II	242	279	301	308	317	NA
		0R318	III	238	273	299	308	320	NA
		0R319	IV	247	290	320	336	356	NA
		0R320	V	234	275	302	316	327	NA
		0R321	VI	231	266	290	306	318	NA
6	150	0R322	VII	240	280	300	310	325	16.3
		0R323	VIII	229	266	284	301	314	15.6
		0R324	IX	241	280	306	327	340	16.3
		0R325	X	244	285	309	332	348	17.2
		0R326	XI	240	276	304	323	345	15.3
		0R327	XII	239	282	306	324	344	15.9
7	0	0R328	XIII	250	286	314	335	358	13.0
		0R329	XIV	250	284	315	338	354	14.4
		0R330	XV	257	287	318	343	368	15.3
		0R331	XVI	236	269	291	307	323	11.9
		0R332	XVII	244	267	290	311	328	12.1
		0R333	XVIII	255	288	317	345	364	14.2
8	30	0R334	XIII	254	290	315	332	355	NA
		0R335	XIV	225	263	286	305	322	NA
		0R336	XV	239	278	300	319	334	NA
		0R337	XVI	249	294	315	332	348	NA
		0R338	XVII	251	288	315	336	352	NA
		0R339	XVIII	245	277	306	327	346	NA
9	150	0R340	XIII	261	286	310	333	351	NA
		0R341	XIV	264	302	328	354	372	NA
		0R342	XV	254	289	300	311	323	NA
		0R343	XVI	233	258	278	293	312	NA
		0R344	XVII	256	291	314	332	351	NA
		0R345	XVIII	231	266	288	314	341	NA

Table 2: Bilateral pupillary reflex data

Dose Group	Daily Dose (mg/kg)	Animal Number	Tail Mark	1st Pupillary Reflex Eexam, date	Right Eye	Left Eye	2nd Pupillary Reflex Eexam, date	Right Eye	Left Eye	3rd Pupillary Reflex Eexam, date	Right Eye	Left Eye
1	0	0R292	I	6/8/10	+	+	6/15/10	+	+	6/22/10	+	+
		0R293	II		+	+		+	+		+	+
		0R294	III		+	+		+	+		+	+
		0R295	IV		+	+		+	+		+	+
		0R296	V		+	+		+	+		+	+
		0R297	VI		+	+		+	+		+	+
2	0	OR298	VII	6/8/10	+	+	6/15/10	+	+	6/22/10	+	+
		0R299	VIII		+	+		+	+		+	+
		0R300	IX		+	+		+	+		+	+
		0R301	X		+	+		+	+		+	+
		0R302	XI		+	+		+	+		+	+
		0R303	XII		+	+		+	+		+	+
3	30	0R304	I	6/8/10	+	+	6/15/10	+	+	6/22/10	+	+
		0R305	II		+	+		-	+		-	-
		0R306	III		+	+		+	+		+	+
		0R307	IV		+	+		+	+		+	+
		0R308	V		+	+		+	+		-	+
		0R309	VI		-	+		-	-		-	-
4	30	0R310	VII	6/8/10	+	+	6/15/10	-	-	6/22/10	-	+
		0R311	VIII		-	+		-	-		-	-
		0R312	IX		+	+		-	-		-	-
		0R313	X		+	+		-	-		-	+
		0R314	XI		+	+		-	+		-	-
		0R315	XII		+	+		-	+		-	-
5	150	0R316	I	6/8/10	+	-	6/15/10	+	+	6/22/10	+	-
		0R317	II		-	+		-	-		+	+
		0R318	III		+	+		+	-		+	-
		0R319	IV		+	+		+	+		+	+
		0R320	V		-	+		-	-		-	+
		0R321	VI		-	+		-	-		-	+
6	150	0R322	VII	6/8/10	+	+	6/15/10	+	+	6/22/10	+	+
		0R323	VIII		-	+		+	+		+	+
		0R324	IX		+	-		+	+		+	+
		0R325	X		-	+		+	-		+	-
		0R326	XI		+	-		+	+		-	+
		0R327	XII		+	+		+	-		+	+
7	0	0R328	XIII	6/9/10	+	+	6/16/10	+	+	6/23/10	+	+
		0R329	XIV		+	+		+	+		-	+
		0R330	XV		+	+		+	+		-	+
		0R331	XVI		+	+		+	+		+	+
		0R332	XVII		+	+		+	+		+	-
		0R333	XVIII		+	+		+	+		-	+
8	30	0R334	XIII	6/9/10	+	+	6/16/10	+	-	6/23/10	-	-
		0R335	XIV		+	+		-	-		-	-
		0R336	XV		+	+		+	-		-	-
		0R337	XVI		+	+		+	-		-	+
		0R338	XVII		-	-		-	-		-	+
		0R339	XVIII		+	+		+	-		-	-
9	150	0R340	XIII	6/9/10	+	-	6/16/10	-	-	6/23/10	-	-
		0R341	XIV		-	-		-	-		-	-
		0R342	XV		-	-		+	-		-	-
		0R343	XVI		-	-		+	+		-	-
		0R344	XVII		+	+		-	+		+	-
		0R345	XVIII		-	+		+	-		-	-

Response = + High Response = ++ Low/No Response = -

Table 3: Data for Serum, liver, and brain PFBA Concentrations

Daily Dose (mg/kg)		Serum [PFBA] at 24 hours post-last dose (ug/mL)	Liver [PFBA] at 24 hours post-last dose (ug/g)	Cortex [PFBA] at 24 hours post-last dose (ug/g)	Cerebellum [PFBA] at 24 hours post-last dose (ug/g)	Rest of the Brain [PFBA] at 24 hours post-last dose (ug/g)	Retinal [PFBA] at 24-hours post-last dose (ng/g)
0	**Mean**	**< LLOQ**	**< LLOQ**	**< LLOQ**	**< LLOQ**	**< LLOQ**	**< LLOQ**
	SD	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
30	**Mean**	**14.25**	**4.46**	**0.32**	**0.33**	**0.28**	**0.12**
	SD	*10.08*	*4.20*	*0.27*	*0.29*	*0.23*	*0.09*
150	**Mean**	**25.08**	**11.20**	**0.83**	**0.85**	**0.82**	**0.32**
	SD	*11.02*	*7.37*	*0.55*	*0.53*	*0.51*	*0.20*

LLOQ for serum, liver, brain, and retina were 5 ng/mL, 25 ng/g, 25 ng/g, and 5 ng/g, respectively.