

Final Report Summary

Client: Institute for Scientific Validation
Protocol #: 2019.05.ST2
Test Compound: TBL-12
Test Compound Receipt Date: 06/19/2019
Experiment Duration: 07/10/2019 – 07/24/2019

Method

30 (15M/15F) Wistar rats, 200g-260g, each received a single oral dose of the test compound (TBL-12). Ten (5M/5F) at a dose of one (1) gram per kilogram body weight, ten (5M/5F) at a dose of five (5) grams per kilogram body weight and ten (5M/5F) at a dose of nine (9) grams per kilogram body weight. Animals were observed for any abnormalities such as respiratory difficulty, ocular or nasal discharge at intervals of 15, 30, 60, 120, and 240 minutes after administration, and daily thereafter for a total of 14 days. All animals survived the 14 day observation period and were then subjected to a necropsy and gross examination with all of the findings noted, The test compound was frozen prior to dosing then thawed and used: diluted 1:9 for 1g/kg, 1:1.8 for 5g/kg, and undiluted at 9g/kg.

Results

Dose (g/kg)	Sex	# Dead/#Dosed	
		(M)	(F)
1.0	5M/5F	0/5	0/5
5.0	5M/5F	0/5	0/5
9.0	5M/5F	0/5	0/5

Under the conditions of this test, no deaths were reported, and all animals were BAR (bright, alert and responsive).

TBL-12 Fixed Dose Study

The purpose of this study was to determine the oral toxicity potential of the test compound, TBL-12, in rats at dose levels of one (1), five (5), and nine (9) grams test compound per kilogram of animal body weight.

Strain: Wistar IGS Rat (Albino)

Vendor: Charles River

Catalog #: 003WISTAR

Sex: equal numbers of males and females

Requested weight: 190-220g

Age at arrival: 6 weeks (Male DOB: 5/16/19, Female DOB: 04/22/19)

Upon receipt, the animals were carefully checked for any abnormalities such as respiratory difficulty, ocular or nasal discharge, dehydration, and/or lethargy.

The subjects were acclimated for 7 days prior to test initiation. They were housed in sterile Individually Ventilated Cages (IVC), 2 to a cage, with contact irradiated Teklad Sani Chip bedding, in a room with a 12-hour light/dark cycle. The room temperature was controlled with an approximate range of 70° to 73° F. Diet consisted of irradiated Teklad global 16% protein rodent diet, as well as water from an automated water system, *ad libitum*.

On 07/09/2019, twenty-four (24) hours prior to test initiation, the rats were weighed, marked, and reexamined for general condition as mentioned above. They were separated into three groups of 5 males and 5 female per group. At 7:00pm food was removed for overnight pre-administration fasting.

On 07/10/2019, at 10:00 am animals were re-weighed for a post fasting weight. A master mix of the TBL-12 and Sterile PBS was prepared according to dosage calculations. Individual doses were, calculated based on post-fast body weight. The dose was administered orally using a 18G, 2mm, 3.0in Cadence Science™ Flexible Plastic (PTFE) Needle with Bite Protector. A new needle was used for each rat. Rats were then returned to their cages, where food and water were made available, *ad libitum*.

Animals were observed at 15, 30, 60, 120, and 240 minutes post-administration and general condition was assessed. Then the animals were weighed and observed at least once daily for a total of 14 days. On day 14 (07/24/2019) all animals were euthanized via Carbon dioxide asphyxiation according to SOP 103.1 and given a complete gross necropsy, with all findings noted.



Observation Log

Table 1

Dose Level: 1g/kg:

Animal Number and Sex	Bodyweight (kilograms) Pre-fast	Bodyweight (kilograms) Post-fast	Minutes:					Days:														Bodyweight (kilograms) Final					
			15	30	60	120	240	1	2	3	4	5	6	7	8	9	10	11	12	13	14						
1F	0.226	0.212	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.248
2F	0.236	0.225	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.267
3F	0.232	0.222	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.261
4F	0.237	0.225	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.270
5F	0.249	0.247	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.291
6M	0.264	0.248	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.340
7M	0.265	0.247	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.333
8M	0.261	0.246	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.343
9M	0.269	0.251	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.333
10M	0.281	0.259	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.357

N = Normal

A = Abnormal

H = Hyperactivity

D = Animal Death

WL = Weight Loss

WG = Weight Gain

Necropsy Comments: Animals 1-4: mild/moderate mottling of the lungs. Animal 5&7: 2mm cortical cyst (cortical-medullary junction) on Kidney. This could be incidental and presumptive. Animals 6, 8-10: No gross changes observed.

Table 2

Dose Level: 5g/kg:

Animal Number and Sex	Bodyweight (kilograms) Pre-fast	Bodyweight (kilograms) Post-fast	Minutes:					Days:														Bodyweight (kilograms) Final			
			15	30	60	120	240	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
1F	0.235	0.226	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.254
2F	0.217	0.210	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.242
3F	0.236	0.223	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.260
4F	0.235	0.218	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.263
5F	0.247	0.235	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.274
6M	0.257	0.237	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.315
7M	0.282	0.259	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.361
8M	0.255	0.242	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.352
9M	0.268	0.256	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.346
10M	0.278	0.254	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.361

N = Normal
A = Abnormal
H = Hyperactivity
D = Animal Death
WL = Weight Loss
WG = Weight Gain

Necropsy Comments: Animals #1-10: No gross changes observed.

Table 3

Dose Level: 9g/kg:

Animal Number and Sex	Bodyweight (kilograms) Pre-fast	Bodyweight (kilograms) Post-fast	Minutes:					Days:														Bodyweight (kilograms) Final		
			15	30	60	120	240	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
1F	0.236	0.224	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.267
2F	0.239	0.226	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.270
3F	0.242	0.231	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.273
4F	0.235	0.223	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.273
5F	0.240	0.229	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.262
6M	0.251	0.231	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.301
7M	0.258	0.242	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.327
8M*	0.255	0.234	N	N	N	N	N	N	N	WL	WL	WL	WG	WG	N	N	N	N	N	N	N	N	N	0.258
9M	0.251	0.235	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.334
10M	0.268	0.249	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0.361

*Bit off the tip of the gavage needle during administration.

N = Normal

A = Abnormal

H = Hyperactivity

D = Animal Death

WL = Weight Loss

WG = Weight Gain

Necropsy Comments: Animals 1&5: mild/moderate mottling of the lungs. Animals: 2-4, 6-7, 9-10: No gross changes observed. Animal 8: Gavage needle present (2.5 inch, 2mm diameter) from the esophagus into stomach. Esophageal sphincter open. Animals 9-10: smaller amounts of ingesta in stomach.

WEIGHT LOG

1g/kg

GROUP	CAGE	ID	9-Jul	10-Jul	11-Jul	12-Jul	13-Jul	14-Jul	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul	22-Jul	23-Jul	24-Jul
1			PRE FAST	POST FAST														FINAL
			11:00 AM	10:00 AM	11:20 AM	9:10 AM	11:00 AM	10:00 AM	9:15AM	8:20 AM	10:00 AM	9:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	8:45 AM	9:00 AM
FEMALES:																		
	1	NOMARK	0.226	0.212	0.224	0.229	0.230	0.230	0.232	0.237	0.236	0.235	0.239	0.243	0.238	0.243	0.241	0.248
	2	MARK	0.236	0.225	0.237	0.244	0.245	0.247	0.252	0.242	0.253	0.260	0.261	0.262	0.256	0.262	0.269	0.267
	2	NO MARK	0.232	0.222	0.235	0.238	0.240	0.248	0.251	0.242	0.253	0.259	0.259	0.252	0.258	0.268	0.268	0.261
	3	MARK	0.237	0.225	0.263	0.244	0.240	0.244	0.251	0.258	0.253	0.252	0.260	0.262	0.259	0.261	0.261	0.270
	3	NO MARK	0.249	0.247	0.241	0.263	0.258	0.264	0.270	0.275	0.264	0.276	0.287	0.287	0.277	0.285	0.291	0.291
MALES:																		
	1	NOMARK	0.264	0.248	0.267	0.271	0.279	0.285	0.293	0.298	0.304	0.311	0.315	0.325	0.322	0.331	0.335	0.340
	2	MARK	0.265	0.247	0.270	0.279	0.278	0.287	0.296	0.300	0.304	0.306	0.312	0.318	0.320	0.324	0.331	0.333
	2	NO MARK	0.261	0.246	0.265	0.278	0.281	0.293	0.303	0.303	0.306	0.312	0.322	0.327	0.327	0.339	0.340	0.343
	3	MARK	0.269	0.251	0.277	0.282	0.281	0.289	0.300	0.300	0.303	0.311	0.315	0.319	0.323	0.332	0.332	0.333
	3	NO MARK	0.281	0.259	0.285	0.289	0.289	0.300	0.310	0.317	0.317	0.327	0.326	0.339	0.342	0.349	0.342	0.357

5g/kg

GROUP	CAGE	ID	9-Jul	10-Jul	11-Jul	12-Jul	13-Jul	14-Jul	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul	22-Jul	23-Jul	24-Jul
2			PRE FAST	POST FAST														FINAL
			11:00 AM	10:00 AM	11:20 AM	9:10 AM	11:00 AM	10:00 AM	9:15 AM	8:20 AM	10:00 AM	9:00 AM	9:00 AM	10:00 AM	4:00 PM	9:05 AM	8:45 AM	9:00 AM
FEMALES:																		
	1	MARK	0.235	0.226	0.240	0.239	0.229	0.238	0.241	0.226	0.235	0.246	0.251	0.247	0.246	0.254	0.256	0.254
	1	NO MARK	0.217	0.210	0.223	0.222	0.224	0.228	0.234	0.240	0.233	0.238	0.238	0.233	0.235	0.242	0.244	0.242
	2	MARK	0.236	0.223	0.238	0.244	0.241	0.244	0.251	0.251	0.248	0.257	0.252	0.261	0.252	0.254	0.260	0.260
	2	NO MARK	0.235	0.218	0.235	0.242	0.236	0.248	0.250	0.248	0.240	0.254	0.260	0.252	0.251	0.262	0.263	0.263
	3	BLK MARK	0.247	0.235	0.256	0.254	0.252	0.257	0.250	0.265	0.265	0.266	0.264	0.269	0.273	0.278	0.279	0.274
MALES:																		
	1	MARK	0.257	0.237	0.260	0.270	0.270	0.277	0.296	0.289	0.287	0.294	0.301	0.301	0.302	0.308	0.312	0.315
	1	NO MARK	0.282	0.259	0.287	0.293	0.299	0.302	0.307	0.320	0.322	0.330	0.339	0.340	0.341	0.348	0.356	0.361
	2	MARK	0.255	0.242	0.261	0.274	0.276	0.287	0.282	0.307	0.303	0.314	0.318	0.324	0.323	0.337	0.341	0.352
	2	NO MARK	0.268	0.256	0.275	0.285	0.282	0.295	0.313	0.310	0.315	0.317	0.324	0.332	0.325	0.338	0.341	0.346
	3	BLK MARK	0.278	0.254	0.270	0.287	0.295	0.303	0.306	0.311	0.316	0.328	0.332	0.340	0.349	0.352	0.357	0.361

9g/kg

GROUP	CAGE	ID	9-Jul	10-Jul	11-Jul	12-Jul	13-Jul	14-Jul	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul	22-Jul	23-Jul	24-Jul
3			PRE FAST	POST FAST														FINAL
			11:00 AM	10:00 AM	11:20 AM	9:10 AM	11:00 AM	10:00 AM	9:15 AM	8:20 AM	10:00 AM	9:00 AM	9:00 AM	10:00 AM	4:00 PM	9:05 AM	8:45 AM	9:00 AM
FEMALES:																		
	1	BLU MARK	0.236	0.224	0.236	0.242	0.239	0.248	0.248	0.258	0.255	0.267	0.260	0.262	0.261	0.267	0.264	0.267
	2	MARK	0.239	0.226	0.242	0.248	0.248	0.255	0.246	0.253	0.259	0.261	0.255	0.263	0.258	0.270	0.264	0.270
	2	NO MARK	0.242	0.231	0.251	0.248	0.253	0.257	0.261	0.257	0.266	0.270	0.268	0.267	0.271	0.276	0.283	0.273
	3	MARK	0.235	0.223	0.241	0.238	0.240	0.246	0.231	0.255	0.243	0.257	0.258	0.262	0.256	0.271	0.270	0.273
	3	NO MARK	0.240	0.229	0.242	0.242	0.241	0.241	0.248	0.251	0.243	0.267	0.254	0.259	0.252	0.255	0.263	0.262
MALES:																		
	1	BLU MARK	0.251	0.231	0.248	0.260	0.256	0.265	0.272	0.272	0.278	0.283	0.285	0.287	0.292	0.298	0.298	0.301
	2	MARK	0.258	0.242	0.263	0.271	0.268	0.274	0.283	0.289	0.287	0.295	0.298	0.304	0.303	0.318	0.322	0.327
	2*	NO MARK	0.255	0.234	0.229	0.213	0.205	0.204	0.206	0.210	0.212	0.216	0.218	0.224	0.233	0.243	0.250	0.258
	3	MARK	0.251	0.235	0.252	0.257	0.266	0.276	0.284	0.287	0.291	0.301	0.308	0.316	0.318	0.328	0.334	0.334
	3	NO MARK	0.268	0.249	0.274	0.284	0.287	0.301	0.312	0.313	0.315	0.324	0.338	0.339	0.336	0.358	0.359	0.361

*BIT OFF GAVAGE NEEDLE TIP

WEIGHT GAIN

	1G	5G	9G
FEMALES:			
	0.036	0.028	0.043
	0.042	0.032	0.044
	0.039	0.037	0.042
	0.045	0.045	0.050
	0.044	0.039	0.033
MALES:			
	0.092	0.078	0.070
	0.086	0.102	0.085
	0.097	0.110	0.024
	0.082	0.090	0.099
	0.098	0.107	0.112