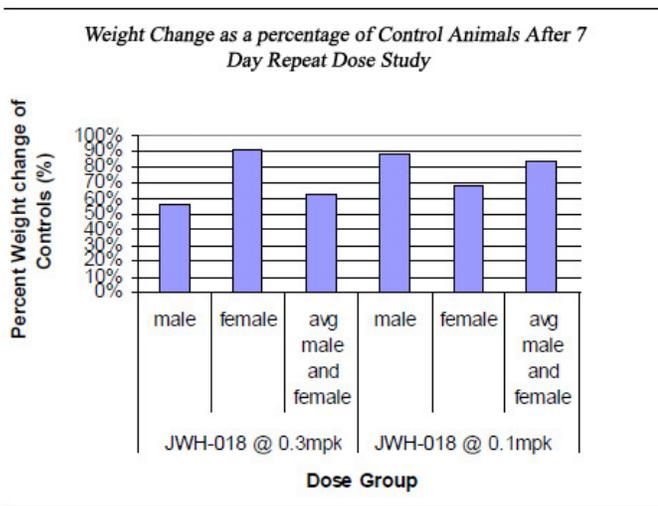


JWH-018 Repeat Rat Toxicity

Repeat Dose Tolerability Testing Clinical Observations:

All animals dosed with JWH-018 appeared lethargic and non-ambulatory up to 2 hrs post dose on day 1, day 2, and day3. At day 4, and day 5 all animals dosed with JWH-018 appeared active, alert, and responsive. On days 6, and 7 all JWH-018 mice appear slightly lethargic for up to 1 hour post dose. All control animals appeared active, alert, and responsive post vehicle administration. The following and in Appendix 1 details the clinical observations as well as weights for all of the animals in this study. There was no tissue abnormalities observed at time of necropsy.

Percent Weight Change After 7 Day Repeat Dose Study as Compared to Control Group:



Individual weights can be found in Appendix 1.

Clinical Observation and Times of Death:

Day 1 –

JWH-018 at 0.3 mg/Kg: Immediately following dose all male and female rats rolled in cage then began to have labored breathing then became lethargic and rigid. Animals began to be active and alert 2 hours post dose.

JWH-018 at 0.1 mg/Kg: Immediately following dose all male and female rats appeared lethargic. All rats began to recover at 15 minutes post dose.

Control: All male and female rats active, alert, and responsive post dose.

Day 2 –

JWH-018 at 0.3 mg/Kg: Immediately following dose all male rats began rolling in cage, then became lethargic for 2 hours post dose. All female rats appeared lethargic and had labored breathing, and then recovered to active and alert 2 hours post dose.

JWH-018 at 0.1 mg/Kg: Immediately following dose all male and female rats appeared lethargic, and then recovered to active and alert 2 hours post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

Day 3 –

JWH-018 at 0.3 mg/Kg: Immediately following dose all male and female rats appeared lethargic, and then recovered to active and alert 1 hour post dose. Male rat # 4 died 1 hour post dose.

JWH-018 at 0.1 mg/Kg: Immediately following dose all male and female rats appeared slightly lethargic, and then recovered to active and alert 1 hour post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

Day 4 –

JWH-018 at 0.3 mg/Kg: All male and female rats appeared active, alert, and responsive post dose.

JWH-018 at 0.1 mg/Kg: All male and female rats appeared active, alert, and responsive post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

Day 5 –

JWH-018 at 0.3 mg/Kg: All male and female rats appeared active, alert, and responsive post dose.

JWH-018 at 0.1 mg/Kg: All male and female rats appeared active, alert, and responsive post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

Day 6 -

JWH-018 at 0.3 mg/Kg: Immediately following dose all male and female rats appeared lethargic, and then recovered to active and alert 1 hour post dose.

JWH-018 at 0.1 mg/Kg: Immediately following dose all male and female rats appeared slightly lethargic, and then recovered to active and alert 1 hour post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

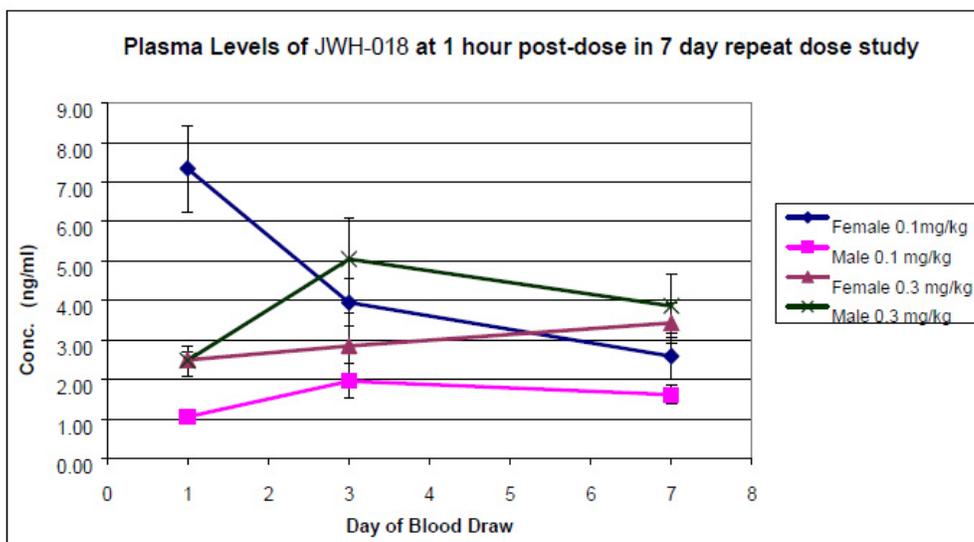
Day 7-

JWH-018 at 0.3 mg/Kg: Immediately following dose all male and female rats appeared lethargic, and then recovered to active and alert 30 minutes post dose.

JWH-018 at 0.1 mg/Kg: Immediately following dose all male and female rats appeared slightly lethargic, and then recovered to active and alert 30 minutes post dose.

Control: All male and female rats appeared active, alert, and responsive post dose.

Plasma Levels of JWH-018 After 1 Hour Post Dose:



Dose (mg/kg)	Sex	Day	Animal	JWH-018 Conc. (ng/ml)	JWH-018 Avg. conc. (ng/ml)	JWH-018 Standard Error Mean							
0.1	F	1	1	5.9									
0.1	F	1	2	9.2									
0.1	F	1	3	5.6									
0.1	F	1	4	14.2									
0.1	F	1	5	7.1									
0.1	F	1	6	6.6									
0.1	F	1	7	7.4									
0.1	F	1	8	4.8									
0.1	F	1	9	10.9									
0.1	F	1	10	1.6	7.3	1.1							
0.1	F	3	1	3.3									
0.1	F	3	2	5.8									
0.1	F	3	3	3.1									
0.1	F	3	4	4.2									
0.1	F	3	5	2.3									
0.1	F	3	6	0.9									
0.1	F	3	7	2.4									
0.1	F	3	8	4.6									
0.1	F	3	9	7.1									
0.1	F	3	10	5.9	4.0	0.6							
0.1	F	7	1	4.9									
0.1	F	7	2	0.6									
0.1	F	7	3	2.2									
0.1	F	7	4	2.6									
0.1	F	7	5	1.0									
0.1	F	7	6	0.8									
0.1	F	7	7	0.6									
0.1	F	7	8	4.2									
0.1	M	3	8	0.2									
0.1	M	3	9	1.3									
0.1	M	3	10	2.3	2.0	0.4							
0.1	M	7	1	1.0									
0.1	M	7	2	1.4									
0.1	M	7	3	1.7									
0.1	M	7	4	1.2									
0.1	M	7	5	2.3									
0.1	M	7	6	1.8									
0.1	M	7	7	3.2									
0.1	M	7	8	0.8									
0.1	M	7	9	2.0									
0.1	M	7	10	1.0	1.6	0.2							
0.3	F	1	1	1.9									
0.3	F	1	2	2.3									
0.3	F	1	3	2.4									
0.3	F	1	4	2.0									
0.3	F	1	5	2.5									
0.3	F	1	6	3.5									
0.3	F	1	7	1.8									
0.3	F	1	8	2.6									
0.3	F	1	9	2.2									
0.3	F	1	10	3.8	2.5	0.2							
0.3	F	3	1	3.9									
0.3	F	3	2	0.3									
0.3	F	3	3	5.7									
0.3	F	3	4	0.2									
0.3	F	3	5	0.3									
0.3	F	3	6	0.2			0.3	M	1	10	1.2	2.5	0.4
0.3	F	3	7	8.0			0.3	M	3	1	9.9		
0.3	F	3	8	3.6			0.3	M	3	2	2.8		
0.3	F	3	9	3.2			0.3	M	3	3	7.2		
0.3	F	3	10	3.1	2.9	0.8	0.3	M	3	4	n/a		
0.3	F	7	1	1.0			0.3	M	3	5	6.9		
0.3	F	7	2	6.7			0.3	M	3	6	5.1		
0.3	F	7	3	2.6			0.3	M	3	7	0.5		
0.3	F	7	4	3.9			0.3	M	3	8	0.7		
0.3	F	7	5	4.4			0.3	M	3	9	5.9		
0.3	F	7	6	4.5			0.3	M	3	10	6.4	5.1	1.0
0.3	F	7	7	3.5			0.3	M	7	1	7.9		
0.3	F	7	8	1.2			0.3	M	7	2	0.6		
0.3	F	7	9	3.4			0.3	M	7	3	2.8		
0.3	F	7	10	3.2	3.4	0.5	0.3	M	7	4	n/a		
0.3	M	1	1	4.7			0.3	M	7	5	2.6		
0.3	M	1	2	2.4			0.3	M	7	6	2.9		
0.3	M	1	3	2.1			0.3	M	7	7	7.3		
0.3	M	1	4	2.9			0.3	M	7	8	5.2		
0.3	M	1	5	2.3			0.3	M	7	9	2.3		
0.3	M	1	6	0.9			0.3	M	7	10	3.1	3.9	0.8
0.3	M	1	7	2.5			vehicle	M+	1, 3, 7	10	<LOD	<LOD	n/a
0.3	M	1	8	4.2			vehicle	M+	1, 3, 7	10	<LOD	<LOD	n/a
0.3	M	1	9	1.5				F	7	10	<LOD	<LOD	n/a

Standard Methods:

Description:

Species: Rat
Strain: Sprague Dawley
Sex: Male and Female
Weight: 205-230 grams

Acquisition and Acclimatization:

Upon receipt, rats were examined by trained personnel to ensure acceptable health status. Rats were acclimated for at least 5 days prior to use.

Method of Identification:

The identification number and compound is displayed on a cage card, along with the rat's date of arrival, date of birth, source, and sex.

Housing:

Rats were housed 3 per cage. Cage size met or exceeded the requirements set forth by the ILAR Guide for the Care and Use of Laboratory Animals. The rats were kept in a room maintained at 64-84oF (22-24oC) with humidity set at 40-70%. The room was illuminated with fluorescent lights timed to give a 12 hour-light, 12 hour-dark cycle.

Feed and Water:

Standard rodent diet (PharmaServ lab diet 5001) and tap water were available for all rats. The feed was analyzed by the supplier, detailing nutritional information and levels of specified contaminants.

Vehicle:

10% ETOH (Sigma Lot# 02343TE)
10% Cremophor EL (Sigma Lot# 037K0213)
80% of 19% hydroxypropyl-beta cyclodextrin in sterile water (Sigma Lot# 048K0672)

Repeat Dose Tolerability Testing:

JWH-018 was dissolved to the appropriate concentration in 10% EtOH, 10% Cremophor EL, and 80% of 19% hydroxypropyl-beta cyclodextrin in sterile water for the IV formulation. Ten male rats and ten female rats were administered an IV dose daily for seven days of 0.3 mg/kg, or 0.1 mg/kg based on body weight for compound JWH-018, and ten male rats and ten female rats were intravenously dosed vehicle. Blood was collected via the venous plexus (retro-orbital) in to tubes containing (K2) EDTA one hour post dose on days 1, 3, 7, on day 8 prior to euthanization. Animals were observed for clinical signs throughout study and euthanized 8 days following the initial compound administration, then tissues removed and stored in formalin for future analysis.

7 Appendix 1

Animal Weights

Animal # & Sex:	Drug & Dose(mg/Kg)	Day 1 Weight: (g)	Dose (ml)	Day 2 Weight: (g)	Dose (ml)	Day 3 Weight: (g)	Dose (ml)	Day 4 Weight: (g)	Dose (ml)	Day 5 Weight: (g)	Dose (ml)	Day 6 Weight: (g)	Dose (ml)	Day 7 Weight: (g)	Dose (ml)	Δ in Weight(g) from Day 0 to Day 7
1 Male	@ 0.3mpk	246	1.64	248	1.68	248	1.68	248	1.68	258	1.74	259	1.74	264	1.78	18
2 Male	@ 0.3mpk	241	1.61	227	1.51	230	1.54	242	1.61	256	1.71	262	1.74	270	1.81	29
3 Male	@ 0.3mpk	231	1.54	230	1.54	226	1.51	234	1.57	247	1.68	250	1.68	252	1.68	21
4 Male	@ 0.3mpk	218	1.47	218	1.47	Dead										0
5 Male	@ 0.3mpk	248	1.68	246	1.64	245	1.64	248	1.68	258	1.74	261	1.74	269	1.81	21
6 Male	@ 0.3mpk	237	1.61	236	1.57	245	1.64	245	1.64	254	1.71	256	1.71	262	1.74	25
7 Male	@ 0.3mpk	231	1.54	231	1.54	237	1.61	239	1.61	239	1.61	235	1.57	247	1.64	16
8 Male	@ 0.3mpk	231	1.54	224	1.51	220	1.47	231	1.54	236	1.57	238	1.61	252	1.68	21
9 Male	@ 0.3mpk	237	1.61	241	1.61	248	1.68	252	1.68	261	1.74	268	1.81	275	1.84	38
10 Male	@ 0.3mpk	240	1.61	233	1.57	238	1.61	247	1.68	255	1.71	260	1.74	284	1.78	24
1 Female	@ 0.3mpk	219	1.47	221	1.47	227	1.54	233	1.54	238	1.61	235	1.57	235	1.57	16
2 Female	@ 0.3mpk	226	1.51	224	1.51	230	1.54	234	1.57	232	1.54	240	1.61	241	1.61	15
3 Female	@ 0.3mpk	219	1.47	211	1.41	220	1.47	222	1.47	225	1.51	224	1.51	224	1.51	5
4 Female	@ 0.3mpk	221	1.47	222	1.47	232	1.54	238	1.61	240	1.61	239	1.61	242	1.61	21
5 Female	@ 0.3mpk	214	1.44	213	1.44	214	1.44	216	1.44	223	1.51	220	1.47	222	1.47	8
6 Female	@ 0.3mpk	229	1.54	226	1.51	232	1.54	235	1.57	238	1.61	226	1.51	239	1.61	10
8 Female	@ 0.1mpk	218	1.47	222	1.47	226	1.54	231	1.54	232	1.54	229	1.54	240	1.61	22
9 Female	@ 0.1mpk	212	1.41	211	1.41	212	1.41	215	1.44	219	1.47	219	1.47	219	1.47	7
10 Female	@ 0.1mpk	210	1.41	211	1.41	215	1.44	221	1.47	229	1.54	232	1.54	226	1.51	16