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2.6.6 TOXICOLOGY WRITTEN SUMMARY

2.6.6.1 Brief Summary

LB Pharmaceuticals, Inc. is developing LB-102, a racemic compound, for the treatment of schizophrenia. The initial clinical study of LB-102 will be a Phase 1 single ascending dose (once daily treatment) and multiple ascending dose (MAD) study assessing safety, tolerability, and pharmacokinetics. The MAD phase will involve twice daily oral dosing for 6.5 days (total of 13 doses).

To support the IND, the following toxicology studies have been conducted on LB-102.

Table 1: Toxicology Program for LB-102

Study Type	Dose Route	Species
Single-dose toxicity	Oral	Rat, dog
Repeat-dose		
14 Days	Oral	Rat, dog
28 Days	Oral	Rat, dog
Genetic toxicity		
Ames	NA	*S. typhimurium*, *E. coli*
In vitro cytogenetics	NA	TK6 cells
NA = Not applicable.		

All of the *in vivo* studies involved oral dosing, the clinically relevant route of administration, in a vehicle of 0.5% methylcellulose (MC). Furthermore, the toxicology studies involved twice daily dosing (BID) in order to simulate the clinical treatment regimen. The studies followed standard toxicological study designs and were compliant with Good Laboratory Practices (GLPs), except the rat and dog range-finding studies which were not required to be GLP-compliant. There were no deviations that impacted the integrity or interpretation of the study data.

Range-finding rat and dog studies were conducted to identify dose ranges for administration in the pivotal 28-day repeat-dose studies. In the range-finding studies, toxicity was observed in both species.

In the comprehensive, GLP-compliant, 28-day, oral repeat-dose toxicity study in rats, doses of 0, 20, 40, and 100 mg/kg/dose (0, 40, 80, and 200 mg/kg/day) were administered BID approximately 12 hours apart with a 1-month recovery. LB-102-related effects in rats were associated with elevated levels of prolactin, which are presumed to occur with LB-102 based on its mechanism of action as a dopamine antagonist. These changes are unique to rodents, have been observed with other dopamine antagonists, were noted at all doses, and included hypertrophied corpora lutea (CLs), decreased CLs, interstitial cell hyperplasia and increased number of atretic follicles in the ovaries, mammary gland lobuloalveolar hyperplasia, and vaginal mucification in females, and mammary gland atrophy and prostatic inflammation in males. Tissue changes either completely resolved or showed a trend to resolution during the recovery period. Given the species-specific nature of the response, the no-observed-adverse-effect-level (NOAEL) was determined to be 200 mg/kg/day, the highest dose administered.

In dogs, the 28-day study used doses of 0, 0.75, 3, and 7.5 mg/kg/dose (0, 1.5, 6 and 15 mg/kg/day) administered BID approximately 12 hours apart; there was a 1-month post-dose recovery period. The main finding was an increase in heart rate at 6 and 15 mg/kg/day. The dogs remained in sinus rhythm and, due to the absence of correlating clinical/veterinary observations, clinical pathology findings, or histopathological findings, this change was not considered to be adverse. Furthermore, no cardiovascular alterations were noted after the recovery period. The NOAEL was determined to be 15 mg/kg/day, the highest dose administered.

In the *in vitro* Ames and micronucleus assays, LB-102 was neither mutagenic, clastogenic, nor aneugenic.

An overview of toxicology studies is provided in Table 2.6.7.1. An overview and summary of the toxicokinetic data are presented in Tables 2.6.7.2 and 2.6.7.3, respectively. A list of the drug lots used in the toxicology studies is included in Table 2.6.7.4.

2.6.6.2 Single-Dose Toxicity

In a non-GLP study, Wistar rats (n = 3/sex/group) were administered single daily doses of 100, 300, 600, and 1200 mg/kg/dose BID approximately 12 hours apart (total daily doses of 200, 600, 1200, and 2400 mg/kg) and the animals were observed for 4 days (MPI Study 2591-001, Table 2.6.7.5). Parameters evaluated included clinical signs (daily), body weight (daily), and food consumption (daily).

On Days 1 or 2 mortality and moribund sacrifice occurred for all animals following single doses of 1200 and 2400 mg/kg. Adverse clinical signs were noted in a few of these animals including decreased activity, ataxia, tremors, recumbency, and/or cold to touch. The only sign in surviving animals was cold to touch in one 600 mg/kg female. There was no effect on body weight or food consumption.

In a non-GLP study, one male and one female Beagle dog were administered single daily doses of 25, 50, 12.5, and 37.5 mg/kg/dose BID approximately 12 hours apart (total daily doses of 50, 100, 25 and 75 mg/kg) with at least a 3-day washout between doses (MPI Study 2591-007, Table 2.6.7.5). Parameters evaluated included detailed clinical signs (twice daily on dosing days, daily on non-dosing days), body weight (daily), and food consumption (daily).

No adverse effects were noted at 25 mg/kg; this dose was considered to be the maximum tolerated dose (MTD). Doses of ≥ 50 mg/kg were not tolerated. Transient weight loss and decreased food consumption occurred at 50 mg/kg. At ≥75 mg/kg, more pronounced body weight loss and decreased food consumption were accompanied by clinical signs of toxicity (decreased activity, salivation, trembling, warm to touch, and/or vomitus).

2.6.6.3 Repeat-Dose Toxicity

2.6.6.3.1 Rats

In the repeat-dose phase of the range-finding study, Wistar rats (n = 5/sex/group) were administered daily doses of 0 (0.5% MC), 50, 100, and 300 mg/kg/dose BID approximately 12 hours apart (total daily doses of 100 and 200 mg/kg/day) for 14 days (MPI Study 2591-001, Table 2.6.7.6). Due to unexpected toxicity and mortality at 300 mg/kg, all animals in this group received the first dose on Day 1 only but were retained for the remainder of the study. Parameters evaluated

included clinical signs (daily), body weight (daily), food consumption (daily), clinical pathology (terminal necropsy), toxicokinetics (TK), gross pathology, and organ weights. The findings from the satellite TK animals are presented in Section 2.6.4.3.2.

Dose formulation analyses revealed a wide range of variability in the preparations as follows: 2.73 to 34.8% of target for the low-dose; -16.2 to 26.7% of target for the mid-dose; and 22.3% of target for the high-dose.

At 300 mg/kg (after the first of the two daily doses on Day 1), three males and one female were moribund sacrificed; a cause of death could not be determined. In addition, two high-dose TK females were found dead or moribund sacrificed on Day 1. Minor effects were generally seen in the animals administered 100 and 200 mg/kg/day. These included: a slight decrease in body weight gain in males at 100 and 200 mg/kg/day; an initial decrease in food consumption in males at 200 mg/kg/day; minor clinical pathology changes (decreased reticulocytes at 100 and 200 mg/kg/day and mildly increased ALT at 200 mg/kg/day); and increases in pituitary weight (males at 100 and 200 mg/kg/day) and prostate weight (males at 100 mg/kg/day).

The MTD was reported to be 200 mg/kg/day.

In the GLP, 28-day repeat-dose study, Wistar rats (n = 16/sex/group) were administered LB-102 orally at doses of 0 (0.5% MC), 20, 40, and 100 mg/kg/dose BID approximately 12 hours apart (total daily doses of 40, 80, and 200 mg/kg/day) (MPI Study 2591-008, Table 2.6.7.7A). Ten rats per sex per group were sacrificed on Day 29 and the remaining 6/sex/group were maintained for a 4-week recovery. Parameters evaluated included detailed clinical signs (daily), body weight (daily), food consumption (daily), ophthalmology (pre-test and at terminal necropsy), clinical pathology (pre-test and at terminal and recovery necropsies), TK, gross pathology, organ weights and histopathology (full tissues for the control and high-dose animals at the terminal necropsy). The heart, mammary gland, adrenal glands, ovaries, vagina, uterus with cervix, and prostate gland were determined to be potential target organs and were examined at the terminal necropsy for the low- and mid-dose animals and for all recovery groups. The TK findings are presented in Section 2.6.4.3.2.

There were no LB-102-related mortalities or adverse test article-related effects on clinical signs, hematology, coagulation, clinical chemistry, urinalysis, or gross pathology. Although minor changes were noted in some of these parameters, they were considered to be non-adverse based on the small magnitude, lack of microscopic correlates, and reversibility.

LB-102-related decreases in body weight were noted in males ≥ 80 mg/kg/day. Body weight gain during the recovery period was similar to or exceeded control values indicating resolution. The effects noted during the treatment period were not considered adverse due to the minor magnitude and the full recovery that was apparent following the 28-day recovery period.

LB-102-related decreases in food consumption were noted in males at ≥ 80 mg/kg/day and females at 200 mg/kg/day. The effects noted during the treatment period were not considered adverse due to the minor magnitude and that full recovery was apparent following the 28-day recovery period.

At the terminal necropsy, several microscopic and organ weight findings were observed which were consistent with elevated levels of prolactin; these are secondary changes. Prolactinemia is a common finding observed with dopamine antagonists, such as LB-102, and these effects in rodents are well documented in the literature. In females at ≥ 40 mg/kg/day, the findings included decreased absolute ovarian weights, hypertrophied corpora lutea (CLs), decreased CLs, interstitial cell hyperplasia and increased number of atretic follicles in the ovaries, mammary gland lobuloalveolar hyperplasia, decreased uterus/cervix weights and vaginal mucification. The ovarian and uterine findings recovered fully, and the mammary changes had decreased in severity at recovery, showing a trend towards reversibility. These findings are specific to rodents when exposed to dopamine antagonists. Furthermore, due to the lack of degenerative findings and the overall similarity to changes normally occurring during pseudo-pregnancy and pregnancy and with other dopamine antagonists, this spectrum of findings was considered to be non-adverse.

Additional findings associated with elevated prolactin levels secondary to the intended pharmacology of LB-102 were noted in males at ≥ 40 mg/kg/day at the terminal necropsy. These included moderate to marked mammary gland atrophy, which exhibited decreased severity at recovery, demonstrating reversibility, and prostatic inflammation and increased prostate weights. The prostatic inflammation persisted through the end of the recovery period. Prostatic inflammation was of low severity, and the mammary gland atrophy was interpreted as non-harmful; thus, the findings in the males are also considered non-adverse.

LB-102 was well tolerated with no adverse findings in any parameter evaluated. Therefore, the NOAEL was considered to be 200 mg/kg/day (associated with Day 28 AUC values of 37200 and 27300 ng•hr/mL in males and females, respectively).

2.6.6.3.2 Dogs

In the 14-day repeat-dose phase of the range-finding study, Beagle dogs (n = 1/sex/group) received oral doses of 0 (0.5% MC), 2.5, 7.5, and 22.5 mg/kg/dose BID approximately 12 hours apart (total daily doses of 5, 15, and 45 mg/kg/day) (MPI Study 2591-007, Table 2.6.7.6). Parameters evaluated included detailed clinical signs (daily), body weight (daily), food consumption (daily), clinical pathology (pre-test and at terminal necropsy), toxicokinetics (TK), gross pathology, and organ weights. The TK findings are presented in Section 2.6.4.3.3.

The primary findings were a dose-related decrease in body weight, body weight gain, and food consumption. The effects at the low-dose were minimal whereas more pronounced/marked effects occurred at the mid- and high-doses. The high-dose dogs required food supplementation throughout most of the study based on the degree of weight loss that occurred. None of the other parameters were affected.

The MTD was determined to be 5 mg/kg/day.

In the GLP, 28-day study, Beagle dogs (n = 5/sex/group) were administered LB-102 orally at dose of 0 (0.5% MC), 0.75, 3 and 7.5 mg/kg/dose BID approximately 12 hours apart (total daily doses of 5, 15 and 45 mg/kg/day) (MPI Study 2591-009, Table 2.6.7.7B). Three dogs per sex per group were sacrificed on Day 29 and the remaining 2/sex/group were maintained for a 4-week recovery. Parameters evaluated included detailed clinical signs (daily), body weight (daily), food consumption (daily), ophthalmology (pre-test and at terminal necropsy), eletrocardiography (pre-

test and at terminal and recovery necropsies), clinical pathology (pre-test and at terminal, and recovery necropsies), TK, gross pathology, organ weights, and histopathology. The TK findings are presented in Section 2.6.4.3.3.

All animals survived to their scheduled terminations. The only clinical sign was occasional emesis/vomitus in two males at 15 mg/kg/day; this was not considered to be adverse as there was no evidence of an adverse effect on the health status of the animals. An initial, slight, dose-related decrease in weight gain or weight loss occurred on Day 4, but by Day 6 animals were gaining weight at a comparable rate as the controls. Decreased food consumption was noted during Week 1 for males at ≥ 6 mg/kg/day. Decreased food consumption generally persisted until Week 3 or through Week 4 for males at 6 and 15 mg/kg/day, respectively. This finding was considered LB-102-related, but was not considered adverse due to the minor magnitude and the lack of correlated test article effects on body weight or evidence of poor health in the animals.

LB-102 was associated with a dose-related increase in the heart rate and shortening of the RR interval at 6 and 15 mg/kg/day on Day 27. The increase in the heart rate was accompanied by a single instance of sinus tachycardia (a normal variant in dogs) for 1 of 5 high-dose males on Day 1 only and a physiologically appropriate shortening of the QT interval. There were no LB-102-related effects on the PR or QTc intervals or QRS duration. These findings were not considered to be adverse as the dogs were still in sinus rhythm and there was a lack of correlating clinical/veterinary observations, clinical pathology findings, or histopathological findings. The quantitative ECG changes were not evident on the recovery ECGs indicating that these findings were reversible.

There were no effects on hematology, coagulation, or urinalysis parameters and only a minimal to mild increase in mean total cholesterol in both sexes at ≥ 6 mg/kg/day on Day 28. Total cholesterol concentrations had resolved following a 28-day recovery period. This finding was not considered adverse due to the minor magnitude and lack of histopathological correlates.

There were no test article-related effects on gross pathology, organ weights, or histopathology.

Based on these data, the NOAEL was considered to be 15 mg/kg/day (associated with Day 28, AUC values of 11800 and 10700 ng•hr/mL in males and females, respectively).

2.6.6.4 Genotoxicity

In vitro GLP- and ICH-compliant genetic toxicity studies have been performed on LB-102.

In the Ames assay, *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were exposed to LB-102 at concentrations of 25 to 5000 µg/plate with and without metabolic activation (Charles River Study 01250001, Table 2.6.7.8A). The concentrations were selected after performing a range-finding assay.

Dose formulation analyses of the low, a mid and the high concentrations revealed values of 82.8, 96.5 and 97.2% of target, respectively. The back-up sample for the low concentration was analyzed and confirmed the low value. There was not a negative impact because the other two concentrations were acceptable and the study was negative. Homogeneity samples confirmed that the dosing materials were homogenous.

LB-102 did not increase the number of revertant colonies at any concentration in any strain with or without metabolic activation, and was concluded to be was negative for inducing mutagenicity in this assay. The positive controls produced the anticipated response.

In the *in vitro* micronucleus assay, TK6 cells were incubated with LB-102 at concentrations ranging from 12 to 384 µg/mL with and without metabolic activation (Charles River Study 01250003, Table 2.6.7.8B). Cells were incubated with drug for 4 hours with and without metabolic activation and for 27 hours without metabolic activation. Due to the lack of toxicity, the three highest concentrations were evaluated for micronuclei (96, 192, 384 µg/mL).

No increase in micronuclei was observed at any dose with or without metabolic activation. The positive controls produced the anticipated response. LB-102 was concluded to be negative for clastogenic and aneugenic activity in this assay.

2.6.6.5 Carcinogenicity

No carcinogenicity studies have been conducted on LB-102 at this time.

2.6.6.6 Reproductive and Developmental Toxicity

No reproductive or developmental toxicity studies have been conducted on LB-102 at this time.

2.6.6.7 Local Tolerance

No local tolerance studies have been conducted on LB-102.

2.6.6.8 Other Toxicity Studies

No other toxicity studies have been conducted on LB-102.

2.6.6.9 Discussion and Conclusions

The toxicity profile for LB-102 has been evaluated in rats and dogs. The types of effects were different for the two species, but the dose-response curve was fairly steep for both. In rats, rodent-specific responses were noted at doses up to and including 200 mg/kg/day (NOAEL); these were associated with prolactinemia and are not considered to be adverse or to represent a safety concern for humans (see discussion below). As the dose increased in this species to 300 mg/kg and above, significant toxicity requiring moribund sacrifice and/or adverse signs or mortality was observed. In dogs, non-adverse changes were noted at doses up to and include 15 mg/kg/day (NOAEL) whereas when the dose was increased to 45 mg/kg/day a marked decrease in body weight and food consumption occurred. Table 2 below presents a comparison of toxicity and the doses and exposures associated with these effects in the two species.

Table 2: Comparison of Toxicity, Doses and Exposures in Nonclinical Species

Dose (mg/kg/day)[a]	Adverse Effects	LB-102 C_{max} (ng/mL)		LB-102 AUC (ng•hr/mL)	
		Male	Female	Male	Female
Rats					
40	None	280 (D28)	202 (D28)	1410 (D28)	1020 (D28)
80	None	1620 (D28)	877 (D28)	7160 (D28)	4230 (D28)
100	Minor BW and FC decreases	2230 (D14)	1150 (D14)	11700 (D14)	7050 (D14)
200	None	***3450 (D14) 5110 (D28)***	***3860 (D14) 4180 (D28)***	***37100 (D14) 37200 (D28)***	***37400 (D14) 27300 (D28)***
300[b]	MS	4940 (D1)	6890 (D1)	91500 (D1)	100000 (D1)
Dogs					
1.5	None	112 (D28)	142 (D28)	789 (D28)	911 (D28)
5	None	343 (D14)	284 (D14)	2100 (D14)	2500 (D14)
6	Minor BW decrease	643 (D28)	614 (D28)	3670 (D28)	3590 (D28)
15	None	***1300 (D14) 1930 (D28***	***1650 (D14) 1810 (D28)***	***8540 (D14) 11800 (D28)***	***8210 (D14) 10700 (D28)***
45	Marked BW and FC decreases	5340 (D14)	3740 (D14)	38100 (D14)	26400 (D14)

BW = Body weight; D = Study day; FC = Food consumption; MS = Moribund sacrifice.
a – The NOAEL and exposure values from the 28-day studies are bolded and italicized.
b – Due to unexpected toxicity and mortality, animals in this group received a single dose of 300 mg/kg on Day 1 only. The data from this group should be interpreted with caution.

The exposure data indicate that for both rats and dogs, significant toxicity occurs at AUC values that are approximately three times higher than the AUC values associated with the NOAEL doses.

The key findings produced by LB-102 in rats were associated with elevated levels of prolactin, which are presumed to occur with LB-102 based on its mechanism of action as a dopamine antagonist. However, the resulting effects of prolactinemia in rodents following LB-102 dosing (e.g. hypertrophied CLs, decreased CLs, interstitial cell hyperplasia, and increased number of atretic follicles in the ovaries, mammary gland lobuloalveolar hyperplasia, and vaginal mucification in females, mammary gland atrophy and prostatic inflammation in males) are unique to that species when dosed with dopamine antagonists and well-known having been observed with other drugs in this class (Krishna et al., 2017; Mostafapour et al., 2014; Ose et al., 2009; Van Coppenolle et al., 2001; Zhang et al., 2015). Much of the species difference is due to the lack of a spontaneous luteal phase for rodents, which humans do have, and the subsequent response to sustained increases in circulating prolactin levels in a manner generally similar to what would be observed following a sterile mating or during a spontaneous pseudopregnancy (Krishna et al., 2017; Perez-Villamil et al., 1992; Rehm et al., 2007). This latter response does not occur in humans, which explains why the rodent hyperprolactinemia findings are not predictive of a safety concern in humans.

To determine, the initial starting dose in humans, the NOAEL doses in both species (200 mg/kg/day in rats and 15 mg/kg/day in dogs) were converted to human equivalent doses (HED) using FDA's 2005 guideline entitled "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." The corresponding doses were 32 and 8.1 mg/kg/day in rats and dogs, respectively. Dogs were the most sensitive species, therefore,

applying a safety factor of 10, the initial starting dose in humans is 0.8 mg/kg/day (approximately equivalent to 50 mg/day for a 60 kg adult).

2.6.6.10 References

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