## Safety Pharmacology

Frequency Therapeutics has conducted three GLP safety pharmacology studies with PROJECT 8 ([Table 4](#_bookmark8)). PROJECT 8 did not alter CNS function in rats at oral doses up to 1000 mg/kg, did not induce any respiratory changes in rats at doses up to 10 mg/kg, and did not induce any cardiovascular changes in conscious dogs up to 50 mg/kg. At these dose levels, assuming 100% systemic absorption of the oral dose, the safety margins ranged from 150 to 15,000 times the intended clinical dose levels of PROJECT 8 on a body surface area comparison basis. Systemic exposures attained in these studies also provide 172 to 17, 241-fold safety margins relative to clinical plasma PK from the Phase 1 study (PROJECT 8-103), in which plasma Cmax for PROJECT 8 following a single intratympanic injection was determined to be 0.58 ng/mL ([Table 4](#_bookmark8)).

## Table 4: Safety Pharmacology Studies with PROJECT 8

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Study Number** | **Species** | **Doses Studied** | **Margin Of Safety (Relative To Human Dose)\*** | | **Summary Results** |
| **Dose Comparison (mg/m2)** | **Exposure Comparison**  **(Cmax)** |
| Potential respiratory effects of orally dosed PROJECT 8 in rats | TR- PROJECT 8- PH-002 | Rat | 0, 10, 100,  1000 mg/kg | 150x | 172x | No-observed effect-level (NOEL) for single-dose oral administration of PROJECT 8 was 10 mg/kg. |
| Potential central nervous system effects of orally dosed PROJECT 8 in  rats | TR- PROJECT 8- PH-001 | Rat | 0, 10, 100,  1000 mg/kg | 15,000x | 17,241x | NOEL for single-dose oral administration of PROJECT 8 was 1000 mg/kg. |
| Potential cardiovascular effects of orally dosed PROJECT 8 in conscious  freely moving male beagle dogs | TR- PROJECT 8- PH-003 | Dog | 0, 1, 10 and  50 mg/kg | 2500x | 5172x | NOEL for single-dose oral administration of PROJECT 8 to conscious, freely moving,  male beagle dogs was considered to be 50 mg/kg. |

* Assuming 100% systemic absorption of oral dose; based on body surface area (mg/m2) comparisons, using Km values of, 6 for rat, 20 for dog and 37 for human (FDA, 2005)

# Toxicology

Frequency Therapeutics has conducted non-GLP and GLP nonclinical single dose, repeat dose and genotoxicity studies for the assessment of potential toxicity as well as for the identification of target organ toxicity ([Table 5](#_bookmark9)). Toxicology studies with the active agent PROJECT 8 and the formulated drug product, PROJECT 8, in rat, dog, and guinea pig indicated a low risk of local and systemic toxicity when administered via intratympanic injection or orally.

## Table 5: Toxicology Program

|  |  |  |  |
| --- | --- | --- | --- |
| **Study type and duration** | **Route of administration** | **Species** | **Compound administered** |
| **Single-dose toxicity**  -Single dose  -Single Dose Escalation  -Single Dose Escalation | Intratympanic Oral  Oral | Guinea Pig Rat  Dog | PROJECT 8 PROJECT 8 PROJECT 8 |
| **Repeat dose toxicity**  -Once a week for 4 doses  -Once/4 days for 4 doses  -Once every 2-4 days  -Once/4 days for 8 doses | Intratympanic Oral  Oral Oral | Guinea Pig Rat  Dog Dog | PROJECT 8 PROJECT 8 PROJECT 8 PROJECT 8 |
| **Genotoxicity**  -Ames  -Chromosomal Aberration  -Micronucleus | In vitro | Salmonella typhimurium histidine auxotrophs TA98, TA100, TA1535 and  TA1537 and Escherichia coli WP2 uvrA | PROJECT 8 |
| In vitro | Mammalian Human Peripheral Blood Lymphocytes | PROJECT 8 |
| In Vivo | Rat Bone Marrow | PROJECT 8 |

In toxicology studies, acute and repeat dose administration of PROJECT 8 by the oral route in the rat and dog elicited no significant toxicity at doses that were 500 to 15,000 times higher on a body surface area basis than the intended maximum clinical dose, assuming 100% systemic absorption ([Table 6](#_bookmark10)). The associated exposure-based safety margins from these studies ranged from 722 to 4172-fold relative to clinical plasma Cmax for PROJECT 8.

## Table 6: Clinical Margin of Safety from Toxicology Studies Conducted With PROJECT 8

|  |  |  |  |
| --- | --- | --- | --- |
| **Toxicology Studies-Systemic Dosing** | | | |
| **Study** | **Test Article/ Route of Administration** | **No Observed Adverse Effect Level (NOAEL)** | **Margin Of Safety Relative To Intended Clinical Dose (PROJECT 8 0.628 mg/dose)\*** |
| **Dose basis (mg/m2)\*** |
| Single Dose Escalation Toxicity in Rat | PROJECT 8/Oral | 1000 mg/kg | 15,000x |
| Single Dose Escalation Toxicity in Dog | PROJECT 8/Oral | 150 mg/kg | 7700x |
| Repeat Dose Toxicity in Rat | PROJECT 8/Oral; One dose every 4 days for 4 doses | 100 mg/kg | 1,500x |
| Repeat Dose Toxicity in Dog | PROJECT 8/Oral; One dose every 4 days for 4 doses | 25 mg/kg | 1,200x |
| Repeat Dose Toxicity in Dog | PROJECT 8/Oral; One dose every 4 days for 8 doses | 10 mg/kg | 500x |
| \* Assuming 100% systemic absorption of oral dose; based on body surface area (mg/m2) comparisons, using Km values of, 6 for rat, 20 for dog and 37 for human (FDA, July 2005) | | | |

In the guinea pig, both single and repeat dose intratympanic injections of PROJECT 8 that provided 2x, 3x and 4x greater doses than the intended clinical doses of both PROJECT 8 and FX00, based on comparison of projected perilymph concentrations, assuming 100% perilymphatic absorption ([Table 7](#_bookmark11)); and 7x, 11x and 16x greater on a surface area (mg/m2) basis, assuming 100% systemic absorption ([Table 8](#_bookmark12)) did not elicit any significant toxico-pathological or functional deficits, and systemic exposure was markedly lower than that observed in the target tissues of perilymph and cochlea.

## Table 7: Clinical Margins of Safety from Intratympanic Toxicology Studies Conducted with PROJECT 8 (Perilymph Concentration Comparison)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Perilymph Volume (µL)** | **Maximum Dose Injected in one ear (mg)** | **Perilymph concentration mg/µL (mM)\*** | **Safety Margin**  **(GP- mg/µL / H- mg/µL)** |
| **PROJECT 8** | | | | |
| Guinea Pig (GP) | 9 µL | Low: 0.069 | 0.008 (16.5) | **2x** |
| Mid: 0.103 | 0.011 (24) | **3x** |
| High: 0.157 mg | 0.017 (37) | **4x** |
| **Human (H)** | **160 µL** | **0.628 mg** | **0.004 (8.6)** | **-** |
| **FX00** | | | | |
| Guinea Pig (GP) | 9 µL | Low: 1.97 | 0.22 (1527) | **2x** |
| Mid: 2.95 | 0.33 (2291) | **3x** |
| High: 4.43 mg | 0.49 (3402) | **4x** |
| **Human (H)** | **160 µL** | **17.72 mg** | **0.11 (769)** | **-** |

\*\*\* Intratympanic Margin of Safety calculations were based on comparisons of perilymph concentrations (assuming 100% absorption of intratympanic dose into perilymph) at the nonclinical NOAEL and at the intended clinical dose, using perilymph volume of 9 µL in Guinea Pig and 160 µL in human (Thorne M, et al., 1999) (Taylor et al., 2002)

## Table 8: Clinical Margins of Safety from Intratympanic Toxicology Studies conducted with PROJECT 8 (Systemic Dose Comparison)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species / Study** | **Dose Level** | **Systemic Dose per Injection (mg)** | | **Systemic Total Body Dose (mg)\*** | | **Margin of Safety Relative to Clinical Dose**  **(Systemic Exposure Comparison)\*\*\*** | |
| **PROJECT 8** | **FX00** | **PROJECT 8** | **FX00** | **PROJECT 8: 0.628**  **mg/dose** | **FX00: 17.72**  **mg/dose** |
| Guinea pig / Single or Repeat Dose Toxicity - PROJECT 8/Bilateral Intratympanic | Low | 0.069 | 1.97 | 0.138 | 3.94 | **7x** | **7x** |
| Mid | 0.103 | 2.95 | 0.206 | 5.9 | **11x** | **11x** |
| High\* | 0.157 | 4.43 | 0.314 | 8.86 | **16x** | **16x** |
| Human / Repeat Dose Clinical Study PROJECT 8/  Unilateral Intratympanic | Clinical Dose | 0.628 | 17.72 | 0.628 | 17.72 | **-** | **-** |

* NOAEL Dose

\*\* Guinea Pigs were dosed by bilateral intratympanic injections

\*\*\* Assuming 100% systemic absorption of bilateral intratympanic dose; based on body surface area (mg/m2) comparisons, using Km values of 8 for guinea pig, and 37 for human. Guinea pig body weight was assumed to be 400 grams, human 60 kg (FDA, 2005)

Clinical plasma PK from the Phase 1 study conducted with a single unilateral intratympanic injection of (0.2 mL) PROJECT 8 ([Table 9](#_bookmark13)) indicated that maximal plasma concentrations (Cmax) of PROJECT 8 (0.58 ng/mL; 0.001 µM) were 27 to 31-fold, and 29 to 178-fold lower than the plasma levels observed at the lowest (0.069 mg), and highest NOAEL dose (0.157 mg) tested in single dose and repeat dose intratympanic guinea pig toxicology studies, respectively. Clinical plasma Cmax for PROJECT 8 was 722 to 4172-fold lower than plasma Cmax levels at the NOAELs or maximum tolerated doses (MTDs) in rat and dog oral toxicity studies with PROJECT 8 ([Table 9](#_bookmark13)).

Plasma Cmax for FX00 in the clinical study (3 µM) was 35 to 46-fold, and 30 to 64-fold lower than the Cmax at the lowest (1.57 mg), and highest dose (4.43 mg) of FX00 tested in single dose and repeat dose intratympanic guinea pig toxicology studies, respectively ([Table 9](#_bookmark13)). Further, the FX00 Cmax in the clinical PK study was 113 to 226-fold lower than the accepted therapeutic range following systemic treatment for epilepsy (50-100 µg/mL; Depacon® Prescribing Information), respectively. FX00 is approved in the US for the treatment of epilepsy and has been extensively studied in healthy volunteers and patients as both oral and intravenous formulations and has a well- documented profile of efficacy and safety/tolerability since its first approval in 1996.

## Table 9: Clinical Margins of Safety from Toxicology Studies Conducted with PROJECT 8 (Systemic Exposure [Cmax] Comparison)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Route of Administration** | | **Doses Tested** | | **Concentration ng/mL (µM)** | | **Margins (relative to clinical Cmax)** | |
| **PROJECT 8** | **FX00** | **PROJECT 8** | **FX00** | **PROJECT 8** | **FX00** |
| **Human** | **PROJECT 8 Single intratympanic dose** | **Clinical Dose** | **0.628**  **mg/dose** | **17.72**  **mg/dose** | **0.58**  **(0.001)**  **(Cmax)** | **441**  **(3.0)**  **(Cmax)** | **-** | - |
| Guinea Pig | PROJECT 8 Single Intratympanic Dose | Low | 0.069  mg/dose | 1.95  mg/dose | 15.5  (0.03) | 15,400  (107) | 27x | 35x |
| Mid | 0.103  mg/dose | 2.95  mg/dose | 18.2  (0.04) | 19,100  (133) | 31x | 43x |
| High | 0.157  mg/dose\* | 4.43  mg/dose\* | 17.4  (0.04) | 20,400  (142) | 30x | 46x |
| Guinea Pig | PROJECT 8-Repeat Intratympanic (4 weekly  doses) | Low | 0.069  mg/dose | 1.95  mg/dose | 16.8  (0.04) | 13300  (92) | 29x | 30x |
| Mid | 0.103  mg/dose | 2.95  mg/dose | 51.3  (0.11) | 17500  (122) | 88x | 40x |
| High | 0.157  mg/dose\* | 4.43  mg/dose\* | 103  (0.22) | 28,200  (196) | 178x | 64x |
| Rat | PROJECT 8-Repeat Oral (one dose every 4 days; 4 doses) | | 100  mg/kg\*\* | - | 1591  (3.4)  (Cmax) | - | 2743x | - |
| Dog | PROJECT 8-Repeat Oral (One dose every 4 days; 4 doses) | | 25  mg/kg\*\* | - | 2420  (5.2)  (Cmax) | - | 4172x | - |
| Dog | PROJECT 8-Repeat Oral (One dose every 4 days; 8 doses) | | 10  mg/kg\* | - | 419  (0.9)  (Cmax) | - | 722x | - |

* NOAEL (No Observed Adverse Effect Level)

\*\* MTD (Maximum Tolerated Dose)

PROJECT 8 was not genotoxic in an in vivo rat micronucleus study (at a systemic dose level that was 31,000x greater that of the intended clinical dose, assuming 100% systemic absorption; mg/m2), in the in vitro Ames bacterial reverse mutation and human peripheral blood lymphocyte chromosomal aberration studies.

Because FX00 is approved for human use in the United States (Depacon® Prescribing Information), and has substantial clinical history since its approval, its safety is well characterized, and Frequency Therapeutics does not plan to conduct any additional safety pharmacology or toxicology studies on this agent alone.

## 5.2.1. Reference Safety Information for Assessment of Expectedness of Serious Adverse Reactions

At this time, there have been no reports of SAEs and therefore, all SAEs reported should be considered as unexpected.