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[redacted] Toxicokinetics in the Rat: Pilot results

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[redacted]

[redacted]

The results presented here are preliminary and should not be considered conclusive. The pilot experiments were a limited exploratory examination of the absorption, distribution, metabolism, and elimination of [redacted] in male and female rats, and these results are strictly to be used for establishing robust test routines for the main study experiments.

Summary of pilot experiments

A pilot study was conducted to determine the absorption, distribution, metabolism, and elimination (ADME) of [redacted] in male and female rats following a single oral dose and a 6-hour dermal exposure at 125 mg/kg. Additionally, the plasma kinetics of [redacted] and selected metabolites were determined following a single oral dose to male and female rats at 125 mg/kg. Selected samples of urine, feces, fat, and plasma from rats in the oral ADME and plasma kinetic experiments were analyzed by radio-chromatography accompanied by liquid chromatography (LC) mass spectrometry (MS) and/or gas chromatography (GC) MS.

1. Oral dose, 125 mg/kg of [redacted] (ADME)

Following a single oral dose at 125 mg/kg and a 168-hour collection-recovery period, the majority of the administered dose was eliminated in the feces (>65%), which contained primarily [redacted] along with minor amounts of [redacted] and an unknown metabolite mass 559 Daltons (Da). Numerous low concentration fluorine-containing metabolites were also detected in feces. Excretion via the urine was a minor elimination pathway with greater than 3-fold more radioactivity recovered in urine from female rats (1.82%) compared to male rats (0.53%). Analysis of urine showed that it contained primarily [redacted] and two unknown metabolites with molecular weights of 202 and 256 Da. Urine from female rats also contained [redacted] an unknown metabolite with a mass of 590 Da, and several other minor metabolites. Elimination of the dose as an organic volatile or as ^{14}C-carbon dioxide was negligible (≤0.30%). At the end of the 168-hour period, terminal tissue concentrations were highest in fat for both male (23.95 µg equiv/g) and female rats (42.29 µg equiv/g). Tissue-to-blood ratios for males were greatest for fat (15.4), liver (8.6), and thyroid (7.8). While in females, fat (61.6), adrenals (23.1), and thyroid (21.2) had the highest tissue-to-blood ratios. LC/MS analysis of fat extracts revealed the presence of [redacted], which appeared to be at higher levels in fat from females compared to males. Additionally, several [redacted] and an unknown compound mass 413 Da were observed in all fat extracts and at approximately the same levels in control rats and rats dosed with [redacted].

2. Dermal exposure, 125 mg/kg of [redacted] (ADME)

Following a single dermal exposure to [redacted], a significant amount of the applied dose (>23%) was removed from the application skin site at the end of the 6-

hour exposure. Greater than 27% of the applied dose volatilized from the application site during the exposure interval; ≤2.01% was eliminated as an organic volatile, with a majority of the elimination occurring 18-hours post-exposure. Negligible levels of the applied dose were recovered in the excreta (<0.1%) or as ^{14}C-carbon dioxide (<0.2%), and tissues taken at terminal sacrifice (168 hours post-dose) did not contain detectable levels of radioactivity, with the exception of the dose skin, which contained ~2 µg equiv/g.

3. Plasma kinetics of [redacted] and selected metabolites following an oral dose, 125 mg/kg of [redacted]

   a. [redacted]

Following a single oral dose of [redacted] to male and female rats at a target of 125 mg/kg, the maximum plasma concentration (C_{max}), which was nearly 2-fold higher in males (541.25 ng/mL) compared to females (248.95 ng/mL), occurred slightly earlier in female rats (T_{max} = 2 hours) compared to male rats (T_{max} = 4 hours). Plasma levels of [redacted] declined rapidly and were not detected beyond 24 hours for either sex. The total systemic exposure or AUC (0-12 hours) was noted to be approximately 2-fold greater for male rats (4455.09 hr•ng/mL) than for female rats (2075.94 hr•ng/mL), and the terminal elimination half-life ranged from 2.52-3.68 hours.

   b. [redacted]

Following a single oral dose of [redacted] to male and female rats at a target of 125 mg/kg, the maximum [redacted] plasma concentration (C_{max}) was slightly higher in males (3.42 µg/mL) compared to females (1.89 µg/mL), and the time to maximum levels occurred earlier in female rats (T_{max} = 5 hours) compared to male rats (T_{max} = 15 hours). [redacted] was not detected in plasma beyond 72 hours for female rats. However, [redacted] was still detectable in plasma from male rats at the end of the sampling period (168 hours). On average, the terminal elimination half-life of [redacted] was >70 hours for male rats and <10 hours for female rats, and the AUC values were noted to be 10-fold greater for male rats (352 hr•µg/mL) than for female rats (32.8 hr•µg/mL).

   c. [redacted]

Following a single oral dose of [redacted] to male and female rats at a target of 125 mg/kg, the maximum [redacted] plasma concentration (C_{max}) was 2.48 and 3.86 µg/mL for male and female rats, respectively. The time to maximum levels (T_{max}) occurred sooner in female rats (1.5 hours) compared to male rats (6 hours). Plasma levels of [redacted] were not detected beyond 24 hours for either sex, and the terminal elimination half-life was 2.24 and 7.80 hours for male and female rats, respectively. Lastly, AUC values were slightly higher for female rats (39.3 hr•µg/mL) compared to male rats (24.5 hr•µg/mL).

d. Additional plasma metabolites

Remaining plasma from dosed rats were pooled and further evaluated by LC/MS. Comparison of the total ion chromatograms from dosed rats to control samples revealed [redacted] and two new unidentified metabolites with molecular weights of 472 and 442 Da.

Conclusions and conduct of main study

The results presented here are preliminary and should not be considered conclusive. The pilot experiments were a limited exploratory examination of the absorption, distribution, metabolism, and elimination of [redacted] in male and female rats, and these results are strictly to be used for establishing robust test routines for the main study experiments.

The results of these limited pilot experiments have demonstrated that [redacted] is systemically available following both oral dosing and dermal exposure. The results also show that [redacted] is metabolized to numerous perfluorinated acids and other yet to be determined metabolites. The plasma kinetic data show that [redacted] are short-lived and that [redacted] has a significantly longer terminal elimination half-life in male rats. Further, examination of plasma revealed the presence of two major metabolites of unknown structure.

Conduct of the main in vivo experiments, incorporating the key learnings from the pilot studies, requires a more robust study design having at least two dose levels, more subjects, the addition of repeat oral and dermal dosing, and the availability of newly synthesized (to-be-proposed) metabolites for quantitative and qualitative use. Also, in order to meet the objectives of the main study, the in vivo results should be accompanied by data from the proposed supplemental in vitro metabolism experiments using rat and human microsomes and hepatocytes.