Evaluation of the Chronic Toxicity and Carcinogenicity of Ammonium 2,3,3,3-Tetrafluoro-2-(Heptafluoropropoxy)-Propanoate (HFPO-DA) in Mice

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Abstract:

Background and Purpose: Subacute and subchronic studies with ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (HFPO-DA; CAS#: 62037-80-3) indicate that the liver is the most sensitive target organ in mice. *In vitro* and *in vivo* mode of action (MOA) analyses, including whole genome transcriptomic analyses, provide overwhelming support for peroxisome proliferator-activated receptor alpha (PPARα) signaling in the mouse liver. It was therefore anticipated that chronic exposure to HFPO-DA would result in liver tumors in mice. At the behest of European regulatory authorities a chronic bioassay in CD-1 mice, designed in compliance with OECD Test Guideline 453, was conducted to assess the chronic toxicity and carcinogenicity of HFPO-DA. In addition, transcriptomic analyses of liver tissue was conducted to assess the MOA for tumor formation.

Methods: Mice (350/sex) were administered 0, 0.05, 0.1, 0.5, or 5 mg/kg-day HFPO-DA in water daily by oral gavage for 9 or 18 months. Full histopathological examinations were conducted at each time point along with clinical chemistry measurements. Unstained liver sections from male and female mice (n = 5 per/dose/sex) were prepared for sequencing of the whole transcriptome according to the TempO-Seq® protocol by BioSpyder Technologies (Carlsbad, California) and HiSeq 2500 Ultra-High-Throughput Sequencing System (Illumina, San Diego, California).

Results: Reduced survival was observed in male mice exposed to 5 mg/kg-day for 18 months but not 9 months. Hepatocellular hypertrophy was the most sensitive histopathological response to HFPO-DA and was observed in both sexes at 9 and 18 months of exposure. Consistent with evidence for a PPARα MOA, liver tumors were increased in males at both timepoints and in females at 18 months. These tumors were significantly increased at 5 mg/kg-day only, resulting in a no-observable-adverse-effect-level (NOAEL) of 0.5 mg/kg-day. No other treatment related tumors were observed. Transcriptomic responses in the liver showed enrichment of PPARα signaling that, consistent with shorter-term studies, indicate that persistent peroxisomal proliferation is responsible for the nonneoplastic and neoplastic lesions in mouse liver. Transcriptomic signatures for other MOAs (e.g., cytotoxicity) were not evident following chronic exposure. Other nonneoplastic changes of interest were increased hypertrophy and weight in the male adrenal cortex at ≥9 months of exposure, and reduced testes weight and decreased germ cell cellularity in the testes after 18 months of exposure. These effects were only significantly increased at 5 mg/kg-day resulting in a NOAEL of 0.5 mg/kg-day. Notably, effects on the male adrenal cortex have been observed in wild type but not PPARα null mice exposed to Wy-14,643.

Conclusions: Overall, chronic exposure of mice to HFPO-DA revealed toxicity to liver of both sexes and to the adrenal cortex and testes of male mice. Transcriptomic analyses from these long term repeat dose studies (9-18 mo) indicate PPARα signaling and are consistent with transcriptomic analyses from much

shorter exposure durations. While the liver effects are clearly adverse to mice in the confines of the study, PPARα-induced liver tumors are well recognized to lack human relevance. The nonneoplastic lesions in the male adrenal cortex and testes may also be secondary to PPARα activation but require more investigation. Data from this study will be included in an updated risk assessment of HFPO-DA.