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Comparison of Phenotypic and Transcriptomic Profiles Between HFPO-DA and Prototypical PPARα, PPARγ, and Cytotoxic Agents in wild-type and PPARα Knockout Mice

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Abstract:

Background and Purpose: The current weight of evidence supports that the subchronic liver effects in rodents following exposure to the short-chain per- and polyfluoroalkyl substances (PFAS) HFPO-DA (ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate; CAS RN 62037-80-3) are consistent with the early Key Events (KEs) of the peroxisome proliferator-activated receptor alpha (PPARα) activator-induced rodent hepatocarcinogenesis mode of action (MOA). The established PPARα MOA consists of four KEs: 1) PPARα activation, 2) alteration in cell growth pathways, 3) perturbation of cell growth and survival, and 4) selective clonal expansion of preneoplastic foci cells. Recent transcriptomic analyses in vitro comparing the transcriptomic profile of HFPO-DA to that of other chemicals with known MOAs further support the PPARα MOA for HFPO-DA-mediated neoplastic and non-neoplastic liver effects in rodents. However, these in vitro analyses primarily address KE 1, PPARα activation, of the PPARα MOA given the absence of nonparenchymal cells to facilitate subsequent KEs involving cell growth, proliferation and survival. Thus, to further inform the MOA for HFPO-DA broadly and determine whether HFPO-DA-mediated liver effects are PPARα-dependent specifically, phenotypic and transcriptomic responses in wild-type (WT) and PPARα knock out (KO) mice were investigated following short-term exposure to HFPO-DA or agents with known MOAs.

Methods: Male WT and PPARα KO mice strains were exposed to various dose levels of HFPO-DA, or well-established agonists of PPARα (GW7647) and PPARγ (rosiglitazone), or hepatotoxicant (acetaminophen [APAP]) for 5 days via oral gavage or for 6 h via intraperitoneal injection (APAP only). Measurements included serum clinal chemistry, liver weight and histopathology, and gene expression by whole-transcriptome templated oligomer sequencing (TempO-Seq) on unstained liver sections.

Results: Whole transcriptomic analyses of mouse livers demonstrated a general lack of transcriptomic response in PPARα KO mice exposed to HFPO-DA or GW7647 based on the low number of differentially expressed genes and enriched gene sets. PPARα KO mice exposed to the highest dose of HFPO-DA (30 mg/kg-d) had a relatively greater transcriptomic response compared to the low- and mid-dose groups; the increased transcriptomic response in the high dose group was unrelated to PPARα-specific genes and pathways indicating an off-target, high-dose response. Importantly, these high dose-related transcriptomic responses were not accompanied by changes in liver weight or histopathology in HFPO-DA-exposed PPARα KO mice. In contrast, WT mice exposed to HFPO-DA or GW7647 had dose-dependent increases in liver weight and increased karyomegaly and mitosis scores via histopathology, as well as enrichment of transcriptomic pathways related to PPARα activation and mitosis. Hepatic transcriptomic responses in rosiglitazone and APAP-exposed mice differed from the responses observed in mice exposed to HFPO-DA or GW7647. A robust enrichment of gene sets related to fatty acid metabolism was observed in PPARα KO mouse livers exposed to rosiglitazone, whereas minimal enrichment was observed in WT

counterparts. Hepatic transcriptomic responses were consistent between APAP-exposed WT and PPARα KO mice, with upregulation of gene biomarkers for cytotoxicity and enriched gene sets primarily related to cell signaling cascades.

Conclusions: The lack of phenotypic and transcriptomic responses in livers from PPARα KO exposed to HFPO-DA supports that the liver effects observed in mice following HFPO-DA exposure are PPARα-dependent. In addition, the phenotypic and transcriptomic responses observed in livers from HFPO-DA-exposed WT mice in this study are consistent with KEs 1-3 of the rodent-specific PPARα MOA; thus HFPO-DA-mediated liver effects in mice are not appropriate endpoints for use in the development of toxicity values for human health risk assessment. This novel study design allows for the investigation of PPARα-dependent and independent mechanisms across several chemicals and can be applied to other PFAS compounds to help inform MOA and human relevance.