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A hypothesis-driven weight-of-evidence analysis to evaluate potential endocrine activity of perfluorohexanoic acid

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ABSTRACT

Perfluorohexanoic acid (PFHxA) is a potential impurity and environmental degradation product of C6-based fluorotelomer products. Considering the potential endocrine activity of perfluoroalkyl acids, a hypothesis-driven weight-of-evidence (WoE) analysis was conducted to evaluate the potential endocrine disruptor activity of PFHxA, as defined by World Health Organization (WHO), across estrogen (E), androgen (A), thyroid (T), and steroidogenesis (S) pathways. A comprehensive literature search identified primary and secondary studies across species for review. The ToxCast/Tox21 database provided *in vitro* data. Studies identified were reviewed for reliability, and relevance, with endocrine endpoints ranked, and lines of evidence evaluated across pathways. Overall, PFHxA showed no endocrine effects in Japanese medaka, juvenile rainbow trout, chickens or reproductive parameters in northern bobwhite with no significant activity in rodent repeated-dose toxicity, lifetime cancer, or reproductive and developmental studies. *In vitro*, there was weak or negative activity for T transport protein or activation of E, A or T receptors. PFHxA was also negative *in vitro* and *in vivo* for disrupting steroidogenesis. Based on this WoE endocrine analysis, PFHxA exposure did not cause adverse effects associated with alterations in endocrine activity in these models, as such would not be characterized as an endocrine disruptor according to the WHO definition.

1. Introduction

The World Health Organization (WHO, 2002) defines endocrine disruptors (EDs) as, “Exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny, or (sub)populations.” Currently, the European Commission (EC) is developing a set of criteria for the identification of such endocrine-disrupting substances that requires regulatory action. At this time, EDs are identified on a case-by-case basis using the available guidance provided in Organization for Economic Co-operation and Development (OECD) Guidance Document 150 (GD 150; OECD, 2012). The OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors provides a tiered framework for organization of study information to assess endocrine activity. This framework is not intended to be a regulatory testing strategy, but to provide guidance in prioritizing relevant data streams and methods according to the type and level of information needed for a regulatory assessment. In the United States, the U.S. Environmental Protection Agency's (EPA's) Endocrine Disruptor Screening Program (EDSP) has been designed to evaluate the endocrine activity of selected substances in a two-tiered testing approach: Tier 1 assays for screening potential endocrine activity, with the focus not on the identification of adverse effects, and Tier 2 studies in non-mammalian and mammalian species that include dose-response changes in apical endpoints to evaluate the potential for a substance to cause adverse responses due to a disruption in a specific endocrine pathway. Both the OECD guidance on evaluating endocrine disrupting properties and the EPA's EDSP are currently under review at this time with changes to focus on data interpretation and alternative methods used to evaluate endocrine activity of a substance.

Perfluorinated compounds are surface active agents that have been used in coatings for packaging, carpets, leather products, and textiles. Among the perfluoroalkyl acids (PFAAs), the 8-carbon (C8) perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been the most extensively studied, and both are currently on EPA's List 2 of substances identified by EPA to be screened for endocrine activity. However, concerns have been raised over the toxicity and

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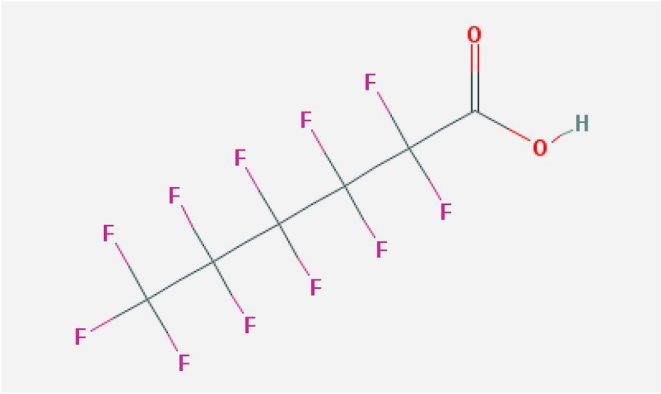

Fig. 1. Structure of PFHxA.

bioaccumulation of these long-chain perfluorinated compounds (C8 and longer), fueling the search for and development of alternative fluorinated compounds consisting of shorter carbon chains. The effort to eliminate the use of PFOA and higher perfluoroalkyl carboxylic acid (PFCA) homologues (USEPA, 2006) has led to the increased use of C6 fluorotelomer compounds that serve as replacements for the C8 compounds (USEPA, 2013).

An impurity, metabolite and or degradation product of C6-fluorotelomer compounds is perfluorohexanoic acid (PFHxA) (CAS No. 307-24-4) (Fig. 1). Levels of PFHxA measured in the environment and in human biomonitoring studies are rarely due to direct exposure to PFHxA itself. PFHxA has been identified in the environment based on the transformation of these mixed-chain-length telomers and also as one of their metabolites identified in exposed mammalian species (Rice, 2015; Russell et al., 2015).

Mulkiewicz et al. (2007) demonstrated *in vitro* that longer perfluorocarbon chain length the greater the toxicity, with PFHxA having the lowest toxicity compared to C8−C10 perfluorinated carboxylic acids. However, based on the potential concern regarding the toxicological effects of PFHxA, a number of guideline studies have been conducted in rats and mice (Chengelis et al., 2009; Klaunig et al., 2015). PFHxA did not cause any reproductive, developmental, or toxic or carcinogenic effects following sub-chronic and chronic repeated-dose administration in rats or mice; however, since there are concerns with polyfluorinated chemicals (PFCs) in general, it was of interest to determine whether there was any potential endocrine activity associated with exposure of humans and/or wildlife to PFHxA. Given the concern regarding endocrine activity of longer-chain-length PFCAs, the fact that fluorotelomer acrylates are metabolized or transformed in the environment to PFHxA, there was a need to evaluate the potential of PFHxA to have endocrine activity, and to determine whether it also has endocrine-disrupting (ED) properties according to the WHO definition (WHO, 2002).

To assess potential ED properties of PFHxA, the published studies in the literature, and available unpublished toxicology study reports, were evaluated to identify endocrine activity of PFHxA across the estrogen (E), androgen (A), thyroid (T), and steroidogenesis (S) pathways. This assessment was conducted by evaluating the endocrine activity of PFHxA in *in vitro* assays and in mammalian and non-mammalian *in vivo* studies, to assess the potential alteration in the function of the endocrine system that results in adverse health effects in an intact organism.

2. Approach and findings

To evaluate the overall endocrine activity of PFHxA, we conducted a comprehensive literature search to capture relevant peer-reviewed literature, along with direct hand-searching to ensure identification of all critical toxicology studies that include endocrine endpoints. Studies are presented according to three categories: *in vitro*, *in vivo* mammalian, and *in vivo* non-mammalian. These studies were reviewed by evaluating their quality and relevance for assessing endocrine activity, along with identifying and extracting endocrine-specific endpoints across the estrogen (E), androgen (A), thyroid (T), and steroidogenesis (S) pathways. *In vitro* data captured from the peer-reviewed literature, as well as high-throughput screening (HTS) assays within the ToxCast/Tox21 database (USEPA, 2016), were integrated into this assessment. Endocrine endpoints extracted from these studies were ranked to facilitate a weight-of-evidence (WoE) analysis across eight endocrine hypotheses, as defined by Borgert et al. (2014) and discussed in Section 3.6 (see also Tables 5–12). The ranking of endocrine endpoints, as defined by Borgert et al. (2014), was based on the strength of these endpoints to interpret the significance of any potential endocrine activity.

Table 1
PubMed- and Embase-specific syntax and numerical results. A PubMed and Embase query using the search string below yielded 119 and 44 results, respectively on January 25, 2017 with a total of 143 results following merging of these databases. An update of the literature search on September 21, 2017, resulted in 15 additional publications considered for review (a total of 158 results). A recent search on May 31, 2018, in Pubmed only, resulted in 19 additional results of which only 1 was carried forward as relevant for full text review.

PubMed
(PFHxA OR "307 24 4"[EC/RN Number] OR (Perfluorinated hexanoic acid) OR ("perfluorohexanoic acid"[Supplementary Concept] OR "perfluorohexanoic acid"[All Fields])) AND (endocrine OR hormone OR estrogen OR estradiol OR estrone OR androgen OR testosterone OR thyroid stimulating hormone OR thyroxine OR aromatase OR thyroid OR steroidogen* OR TSH OR triiodothyronine OR gonad OR (receptor AND binding) OR reproduct* OR development* OR sperm OR testes OR testis OR ovary OR ovaries OR offspring OR neurotox* OR cancer OR carcinogen* OR bioassay OR (repeat* AND dose) OR toxic* OR ecotox* OR zebrafish OR minnow OR medaka OR mammalian OR amphibian OR fish OR non-mammalian OR (gonadosomatic AND indexes) OR vitellogenin OR (vtg AND "production") OR (receptor AND gene AND expression) OR (competitive AND binding))
Embase (https://www.embase.com/)
(pfhxa OR '307 24 4':rn OR (perfluorinated AND hexanoic AND acid) OR 'perfluorohexanoic acid') AND (endocrine OR hormone OR estrogen OR estradiol OR estrone OR androgen OR testosterone OR thyroid AND stimulating AND hormone OR thyroxine OR aromatase OR thyroid OR steroidogen* OR tsh OR triiodothyronine OR gonad OR (receptor AND binding) OR reproduct* OR development* OR sperm OR testes OR testis OR ovary OR ovaries OR offspring OR neurotox* OR cancer OR carcinogen* OR bioassay OR (repeat* AND dose) OR toxic* OR ecotox* OR zebrafish OR minnow OR medaka OR mammalian OR amphibian OR fish OR 'non mammalian' OR (gonadosomatic AND index*) OR vitellogenin OR (vtg AND 'production') OR (receptor AND gene AND expression) OR (competitive AND binding)) AND ([embase]/lim NOT [medline]/lim)

2.1. Peer-reviewed literature search and study identification

A comprehensive literature search was conducted to confirm that all relevant *in vitro* and *in vivo* mammalian and non-mammalian (ecotoxicology) studies in the peer-reviewed literature would be captured to evaluate the endocrine activity of PFHxA (Table 1). To execute the search, syntax unique to each database queried (i.e., PubMed and Embase) was developed in which all relevant chemical identification (e.g., synonyms and registry numbers), endocrine pathways, general toxicity, and carcinogenesis key words were captured. This syntax was reviewed by endocrine and ecotoxicology experts prior to implementation of the search.

The resulting published literature was reviewed and organized to meet two objectives: (1) To identify studies reporting data relevant to the evaluation of PFHxA in the context of the E, A, T, and S pathways (primary objective), and (2) identify studies that contain contextual information relevant to the primary objective, such as toxicokinetics, review articles, and evaluations of similar substances (secondary