

Effects of Perfluorinated Phosphate Derivatives on Gene Expression in the H4IIE Rat Hepatoma Cell Line

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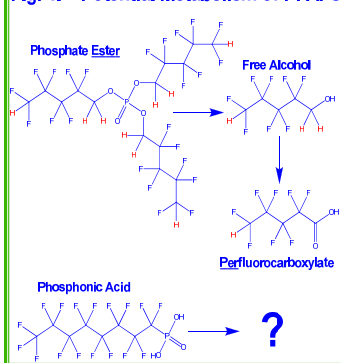
ABSTRACT

Perfluorinated chemicals (PFCs) have gained much recent notoriety due to widespread occurrence in the environment and wildlife and their toxicological effects. Recognition of the potential adverse effects of PFCs has led to development and use of a variety of alternative compounds, some still containing perfluorinated moieties. Notable among these alternative chemicals are the perfluoroalkyl-phosphates (PAPs). These compounds are either the phosphonic acid analogs of carboxylic acids or are mono-, di- or tri-phosphate esters, commonly of fluorotelomer alcohols. During environmental and metabolic breakdown PAPs can be expected to yield a variety of perfluorinated moieties including the parent phosphonic acids and compounds such as FTOHs and potentially perfluorocarboxylates after further degradation of those FTOHs. The objectives of the current study were to determine if PAPs caused the same changes in gene expression as PFCs and whether the gene expression effects of some PAPs were the same as the effects caused by the constituent PFC moieties. Rat hepatoma cells were treated with different PAPs and PFCs at concentrations of 1.0-100 uM for 24 and 72 hrs. RNA was isolated, purified and gene expression was quantitatively measured using Real-Time PCR. Processes investigated included fatty acid synthesis, cellular communication, and thyroid development. Perfluorophosphonic caused differential gene expression profiles relative to analogous perfluorocarboxylates. For example PFDDPA at 10 uM caused 69-fold induction of Apo A4 while the analogous PFDA caused only 6.4 fold induction of the same gene. Effects of phosphate ester compounds were different from comparable perfluoroalcohols which would be expected to be released by metabolic breakdown. We therefore conclude that PAPs may act through mechanisms of action different from other PFCs and so greater research and understanding of the effects of this emerging chemical group is required.

INTRODUCTION

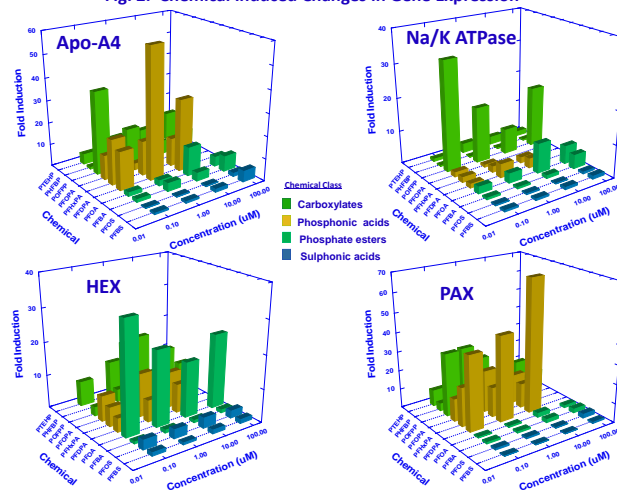
- Perfluorinated compounds (PFCs) have been produced and used in large quantities since the 1950s. While some of the predominant compounds are no longer produced the search for alternative perfluorinated chemicals continues.
- Perfluoroalkyl phosphates (PFAPs), analogues of the sulphonic and carboxylic acid PFCs have recently been identified in the environment (D'eon et al 2009 *Environ. Toxicol. Chem.* 28:2101-2107).
- Currently two classes of PFAPs are of interest; Perfluoroalkyl phosphate esters (PEs) and the phosphonic acids (PAs).
- PEs have the potential to release perfluoro moieties when hydrolysed. These moieties may be metabolized to carboxylates in organisms (Fig. 1).
- Phosphonic acids may possess unique toxic potential due to the presence of the C-P bond.
- In this study PEs and PAs were compared to other PFCs (Table 1) with respect to their ability to modulate the expression of several genes.
- Genes were selected based on the known effects of other PFCs and on their ability to act as markers for critical biochemical functions (Table 2).

Fig. 1. Potential Metabolism of PFAPs



RESULTS

Fig. 2. Chemical Induced Changes in Gene Expression



RESULTS

Fig. 3. Chemical Ordination Based on Gene Expression

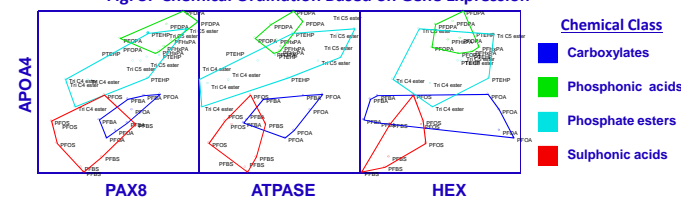
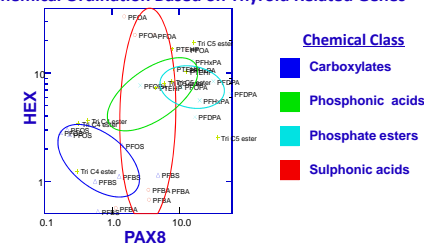
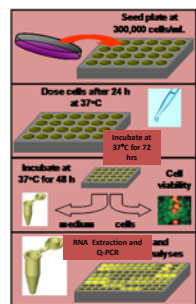


Fig. 4. Chemical Ordination Based on Thyroid Related Genes



METHODS

- Rat hepatoma cells (H4IIE cell line) were cultured under standard conditions, 37°C, 5% CO₂.
- 24 hrs after plating exposure chemicals were added, cell were incubated for 72 hrs.
- After 72 hrs mRNA was extracted from cultured cells and stored in 'RNAlater' until analysis.
- RT-PCR was performed using standard methods, all expression values were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase as a house keeping gene.



- Cell culture and exposure (24 and 72 hrs)
- RNA Extraction
- cDNA Synthesis
- Q-PCR
- Normalize to G3P-DH

CONCLUSIONS

- PFAPs of both classes induced greater changes in Apo-A4 expression than any of the carboxylic and sulphonic acids tested (Fig. 2). Increased expression of Apo-A4 has been linked with decreases in feeding responses, and is believed to be a vital signal for lipid uptake, transportation and metabolism.
- Of the phosphonic acids tested the C10 (PFDDPA) was generally the most potent for alterations in gene expression analogous of the greater potency of the C8-10 PFCs.
- POFPP (C5 ester) induced increased the expression of Na⁺/K⁺ ATPase even at the lowest dose tested and was clearly the most potent chemical for this endpoint (Fig. 2). This transporter is involved in osmotic homeostasis this response may be indicative of alterations in membrane permeability.
- The expression profile of the phosphate esters overlaps or 'approaches' that of the carboxylates suggesting that the metabolic generation of carboxylates may be occurring.
- This study clearly demonstrates that PFAPs elicit a range of effects on gene expression that are distinct from those elicited by other PFCs (Fig. 3). Therefore, further studies on the potential modes of action of these emerging contaminants is warranted.
- Alteration of PAX 8 and HEX expression by PFAPs, particularly the phosphonic acids (PAs) may be indicative of potential impacts on thyroid function and homeostasis (Fig. 4).

Acknowledgements

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Table 1 Chemicals Tested for Effects on Gene Expression

Abbreviation	Type	Name	Chain Length
PFBS	Sulphonic Acid	Perfluorobutyl sulphonic acid	C4
PFOS	Sulphonic Acid	Perfluorooctyl sulphonic acid	C8
PFBA	Carboxylic Acid	Perfluorobutyl carboxylic Acid	C4
PFOA	Carboxylic Acid	Perfluorooctyl carboxylic Acid	C8
PFOPA	Phosphonic Acid	Perfluoroethyl phosphonic acid	C8
PFDDPA	Phosphonic Acid	Perfluorodecyl phosphonic acid	C10
PFHxPA	Phosphonic Acid	Perfluorohexyl phosphonic acid	C6
POFPP	Phosphate Ester	Tris (octafluoropentyl) phosphate	C5
PHFBP	Phosphate Ester	Tris (heptafluorobutyl) phosphate	C4
PTEHP	Phosphate Ester	Tris (trifluoroethyl) phosphate	C2

Table 2 Genes Investigated (responses normalized to Glyceraldehyde-3-phosphate dehydrogenase)

Gene	Function
Na ⁺ /K ⁺ ATPASE	Maintenance of cellular osmotic balance
PAX 8 (Paired box gene 8)	Related to thyroid follicular cell development
HEX (Homeobox)	Related to thyroid cell differentiation
Apo-A4 (Apolipoprotein A-IV)	Related to fatty acid metabolism