

ehp

**ENVIRONMENTAL
HEALTH
PERSPECTIVES**

ehponline.org

**Genomic Profiling Reveals an Alternate
Mechanism for Hepatic Tumor Promotion by
Perfluorooctanoic Acid in Rainbow Trout**

**Susan C. Tilton, Gayle A. Orner, Abby D. Benninghoff,
Hillary M. Carpenter, Jerry D. Hendricks,
Cliff B. Pereira, and David E. Williams**

**doi:10.1289/ehp.11190 (available at <http://dx.doi.org/>)
Online 9 May 2008**



NIEHS
National Institute of
Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Genomic Profiling Reveals an Alternate Mechanism for Hepatic Tumor Promotion by Perfluorooctanoic Acid in Rainbow Trout

Susan C. Tilton^{1,2,3,*}, Gayle A. Orner³, Abby D. Benninghoff^{1,2,4}, Hillary M. Carpenter^{1,†}, Jerry D. Hendricks^{1,2}, Cliff B. Pereira^{4,5} and David E. Williams^{1,2,3,4}

¹Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR

²Marine and Freshwater Biomedical Sciences Center, Oregon State University, Corvallis, OR

³Linus Pauling Institute, Oregon State University, Corvallis, OR

⁴Environmental Health Sciences Center, Oregon State University, Corvallis, OR

⁵Department of Statistics, Oregon State University, Corvallis, OR

[†]Present address: Minnesota Department of Health, St. Paul, MN, USA

*To whom correspondence should be addressed (Present address): Susan C. Tilton, Fred Hutchinson Cancer Research Center, P.O Box 19024, 1100 Fairview Ave. N, C1-015, Seattle, WA, 98109. Tel: +1 206 647-4074; Fax: +1 206 647-5815; Email: stilton@fhcrc.org

Acknowledgements: The authors wish to thank Eric Johnson, Greg Gonnerman, Dan Arbogast and Ted Will for care and maintenance of fish, Jean Barnhill and Connie Owston for histological preparation. This work was supported by NIH grants ES07060, ES03850, ES00210, ES11267, ES013534.

Running Title: Tumor Promotion by PFOA

Key words: clofibrate, dehydroepiandrosterone, estradiol, hepatocarcinogenesis, microarray, perfluorooctanoic acid, peroxisome proliferation, rainbow trout

Abbreviations:

AFB ₁	aflatoxin B ₁
BF	basophilic foci
CathD	Cathepsin D
CCC	cholangiocellular carcinoma
Ch	cholangioma
CLOF	clofibrate
CYP1A	cytochrome P450 family 1A
CYP2K1	cytochrome P450 family 2K1
CYP2K5	cytochrome P450 family 2K5
DFCI	Dana Farber Cancer Institute
DHEA	dehydroepiandrosterone
E2	17 β -estradiol

EIA	enzyme immunosorbent assay
ELISA	enzyme linked immunosorbent assay
ER α	estrogen receptor alpha
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPX	glutathione peroxidase
HCA	hepatocellular adenoma
HCC	hepatocellular carcinoma
MA	mixed adenoma
MC	mixed carcinoma
OTD	Oregon test diet
PCNA	proliferating cell nuclear antigen
PFOA	perfluorooctanoic acid
PP	peroxisome proliferator
PPAR α	peroxisome proliferator-activated receptor alpha
VTG	vitellogenin

Outline:

Abstract

Introduction

Materials and Methods

Materials and animals

Tumor experiment, necropsy and histopathology

Microarray experiment

Peroxisomal β -oxidation and catalase activity

Serum VTG and E2

Microarray hybridization and analysis

Real time qRT-PCR

Results

Tumor study

Gene expression analysis

Discussion

Conclusions

References

Tables

Figure Legends

Figures

Abstract

Background: Perfluorooctanoic acid (PFOA) is a potent hepatocarcinogen and peroxisome proliferator (PP) in rodents. Humans are not susceptible to peroxisome proliferation and are considered refractory to carcinogenesis by PPs. Previous studies with rainbow trout indicate they are also insensitive to peroxisome proliferation by the PP, dehydroepiandrosterone (DHEA), but are still susceptible to enhanced hepatocarcinogenesis after chronic exposure.

Objectives: In this study, we utilized trout as a unique *in vivo* tumor model to study the potential for PFOA carcinogenesis in the absence of peroxisome proliferation compared to structurally diverse PPs, clofibrate (CLOF) and DHEA. Mechanisms of carcinogenesis were identified from hepatic gene expression profiles phenotypically anchored to tumor outcome.

Methods: Aflatoxin B₁ or sham-initiated animals were fed 200-1800 ppm PFOA in the diet for 30 weeks for tumor analysis. Gene expression was subsequently examined by cDNA array in animals fed PFOA, DHEA, CLOF or 5 ppm 17 β -estradiol (E2; a known tumor promotor) in the diet for 14 days.

Results: PFOA (1800 ppm or 50 mg/kg/day) and DHEA treatments resulted in enhanced liver tumor incidence and multiplicity ($P < 0.0001$) while CLOF showed no effect. Carcinogenesis was independent of peroxisome proliferation measured by lack of peroxisomal β -oxidation and catalase activity. Alternately, both tumor promoters, PFOA and DHEA, resulted in estrogenic gene signatures with strong correlation to E2 by Pearson correlation ($R = 0.81$ and 0.78 , respectively), while CLOF regulated no genes in common with E2.

Conclusions: These data suggest the tumor promoting activities of PFOA in trout are due to novel mechanisms involving estrogenic signaling and are independent of peroxisome proliferation.

Introduction

Perfluorooctanoic acid (PFOA) is a member of a class of perfluorinated compounds, which are widely used in consumer products and industrial applications including surfactants, lubricants, textile coatings, food packaging and flame retardants. PFOA is also a degradation product of other fluoropolymers that is highly resistant to further metabolic and environmental breakdown. Due to its widespread occurrence and chemical stability, there are increasing concerns about the environmental persistence and accumulation of PFOA measured in terrestrial and aquatic biota and in human serum (Calafat et al. 2007; Houde et al. 2006; Prevedouros et al. 2006). Estimation of PFOA half-lives in serum vary broadly depending on species and gender ranging from days in rat (Vanden Heuvel et al. 1991) and *Cynomolgus* monkeys (Butenhoff et al. 2004) to almost 4 years in occupationally exposed humans (Olsen et al. 2007). While some perfluorinated chemicals have been voluntarily removed from the market by manufacturers over concerns related to environmental occurrence and stability, PFOA is still produced commercially, and its potential risk to humans continues to be evaluated (U.S. EPA 2006).

PFOA is a potent peroxisome proliferator (PP) similar to other perfluorinated chemicals (Sohlenius et al. 1992). Overall, PPs comprise a structurally diverse group of non-genotoxic carcinogens including certain hypolipidemic drugs (clofibrate, ciprofibrate), industrial plasticizers (phthalates), herbicides (phenoxyacetic acids) and organic solvents (trichloroethylene). They are known to cause hepatomegaly, altered cholesterol homeostasis, increased number and size of peroxisomes and increased β - and ω -oxidation of fatty acids in peroxisomes and microsomes, respectively, in susceptible animal models (Moody et al. 1991). Prolonged exposure to PPs, including PFOA, also results in increased liver tumor incidence in