Transcriptomic Sensitivity of Acute and Chronic *In Vitro* Exposure to Perfluorooctanoic Acid in Human Vascular Endothelial Cells: Insights from Benchmark Concentration Modeling

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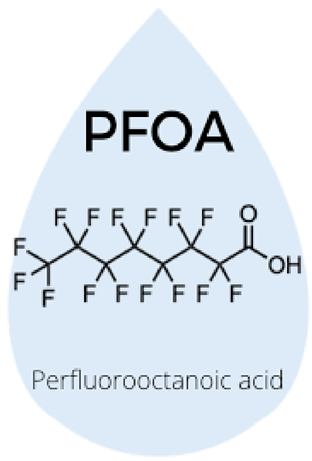
INTRODUCTION

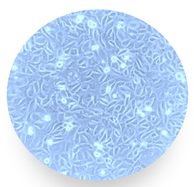
Perfluorooctanoic acid (PFOA), a synthetic chemical known for its persistence in the environment, is commonly used in various industrial and consumer products. PFOA has been linked to a range of adverse health effects, including reproductive and developmental toxicity, hepatotoxicity, renal toxicity, etc. However, the effects of PFOA on human vascular endothelial cells remain poorly understood, particularly in terms of sensitive time points where its impact may be most pronounced.



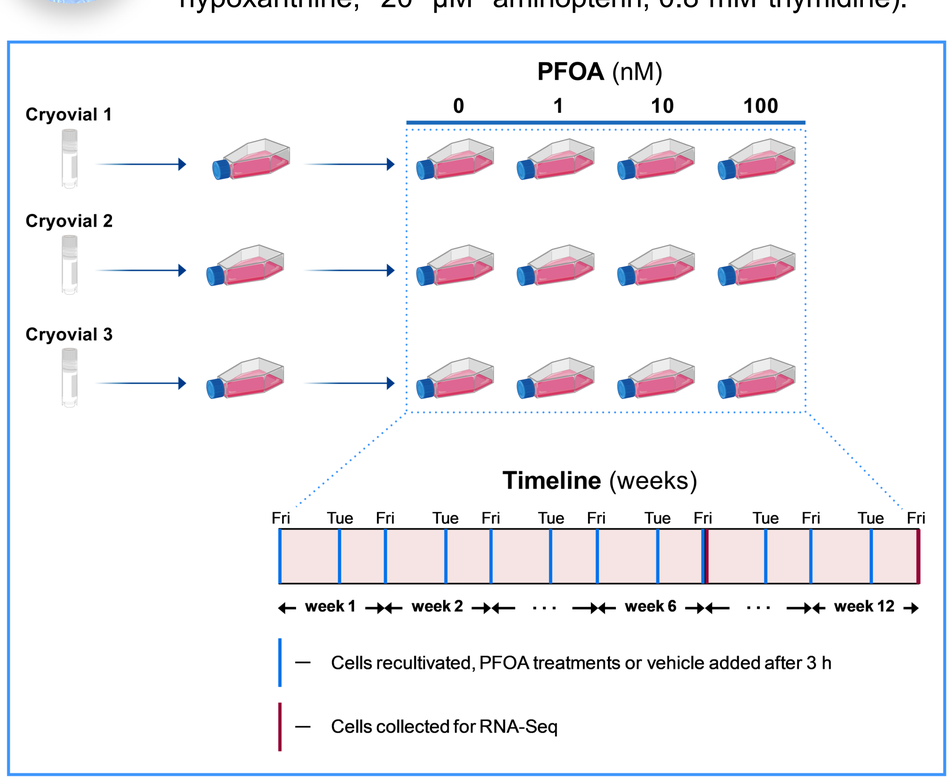
“forever chemical”

The goal of this study was to employ benchmark concentration (BMC) modeling to derive and compare points of departure (POD) for transcriptomic changes (T-POD) in human vascular endothelial cells following short-term (48 h, acute) and long-term (6 and 12 weeks, chronic) exposure to 1, 10, and 100 µM and 1, 10, and 100 nM PFOA, respectively.

METHODS



EA.hy926 cells → grown at 37 °C in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, 1.5 g/L $NaHCO_3$, 0.11 g/L sodium pyruvate, 10 mM HEPES, and 4% HAT (5 mM sodium hypoxanthine, 20 µM aminopterin, 0.8 mM thymidine).



RNA-Seq – BGI Europe (Warsaw, Poland), on the DNBSEQ platform, with sequencing length PE150 and an average yield of 6.65G data per sample. All samples had a RIN>9 and were of very high quality. Sample reads were trimmed to remove any reads with more than 5% unknown base content, along with adapters and low-quality bases. HISAT was used to align the clean reads to the reference genome, while Bowtie2 was used to align the clean reads to reference genes. The analysis of differentially expressed genes (DEG) was performed using the DESeq2 method. The genes with |log2|>1 and adjusted *p*-value of <0.05 were considered as DEGs.

BMC modeling was conducted on log2-transformed DESeq2 normalized gene counts using the BMDExpress3 available at https://github.com/auerbachs/BMDExpress-3. Functional analysis of genes with calculated BMCLs (PODs) was performed using DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/home.jsp).

CONCLUSIONS

The highest number of DEGs after 6 weeks of exposure, with 10 nM PFOA yielding the greatest total number of DEGs.

T-POD values for 6 and 12 weeks indicate greater sensitivity than the T-POD value derived from 48-h exposure – more informative.

Functional gene analysis revealed that transcription was a sensitive, yet general molecular pathway affected by short-term exposure.

6 weeks – IL-17 signaling pathway was notably enriched.

12 weeks – pathways related to extracellular matrix, cytokine-cytokine interactions, and cell adhesion were significantly affected.

RESULTS

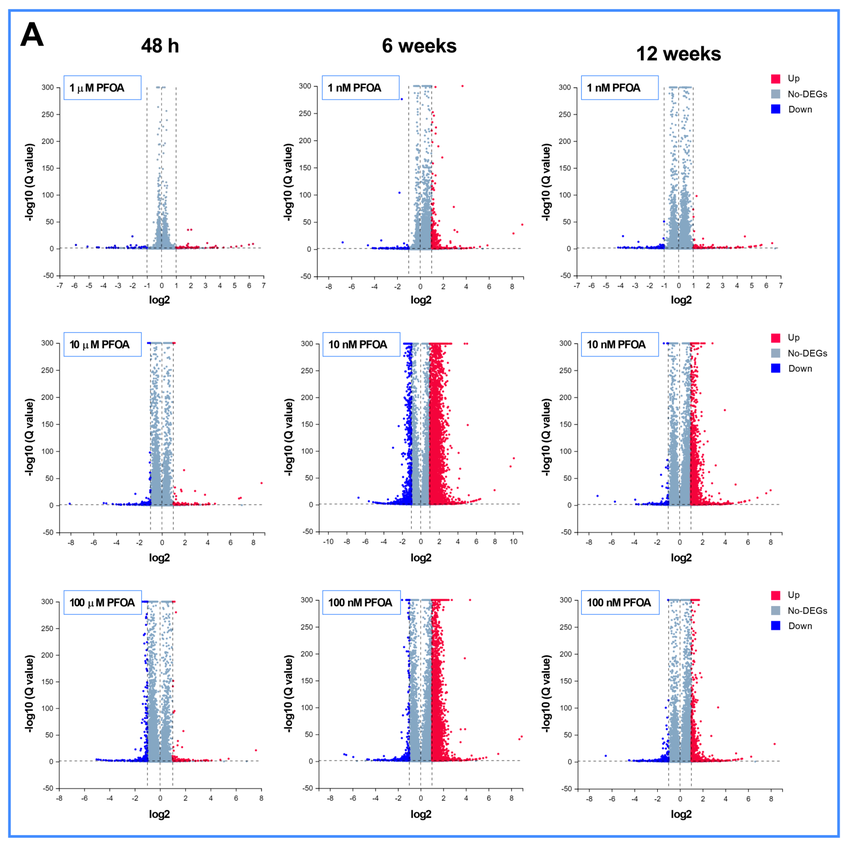


Figure 1. Volcano scatter plots depicting global mRNA expression and the number of DEGs following short-term (48 h) and long-term (6 and 12 weeks) exposure of EA.hy926 cells to specified concentrations of PFOA. **(A)** The X-axis represents the fold change of the difference after conversion to log2, whereas the Y-axis represents the significance value (Q value) after conversion to –log10. In each plot, significantly upregulated genes are marked as red dots, downregulated genes as blue dots, and non-significant findings as gray dots. **(B)** The X-axis displays the number of DEGs, and the Y-axis shows the different concentrations of PFOA. For each concentration, red bars represent the number of upregulated genes, while blue bars represent the number of downregulated genes.

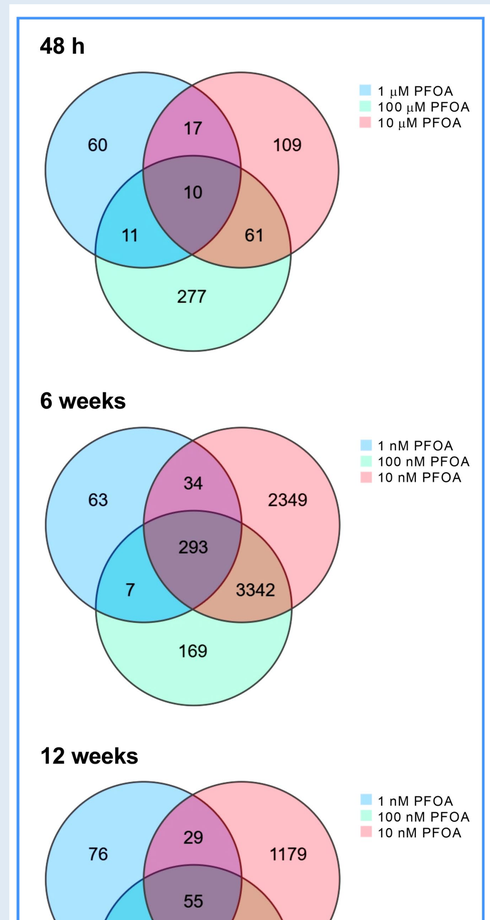


Figure 2. Venn diagram illustrating DEGs after short-term (48 h) and long-term (6 and 12 weeks) exposure of EA.hy926 cells to PFOA. The diagram highlights the number of unique and overlapping DEGs among the specified concentrations of PFOA.

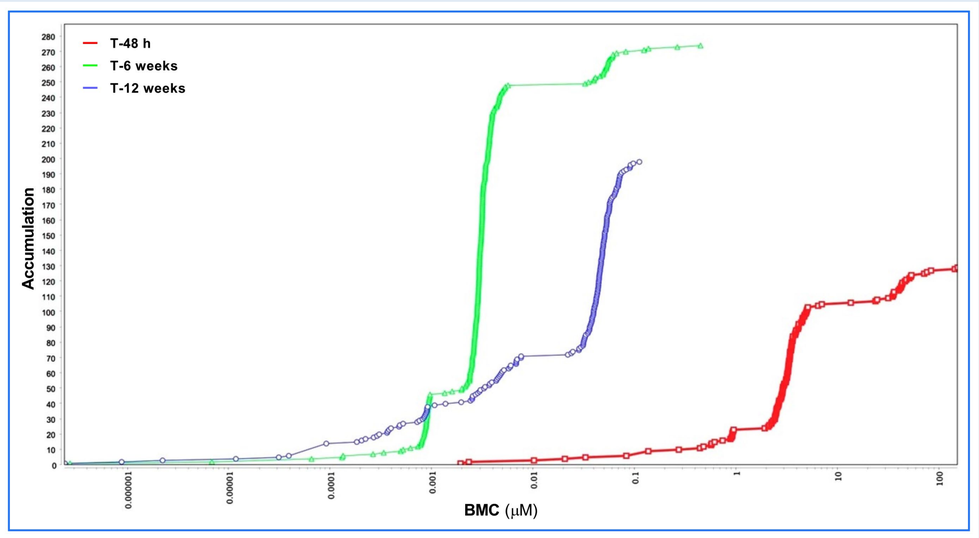
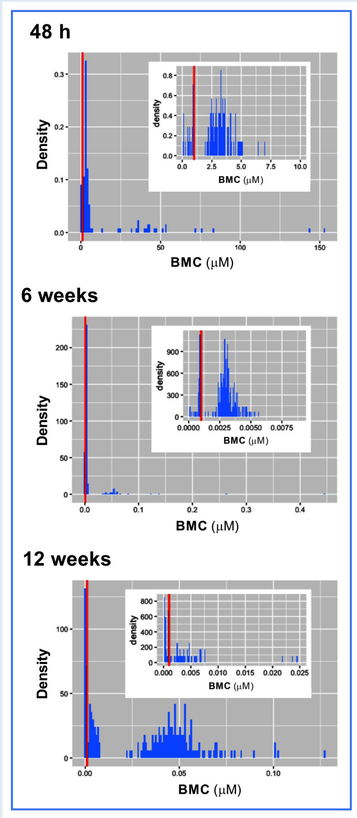


Figure 3. Accumulation plots of benchmark concentration (BMC) values for transcriptomics (T) data following short-term (48 h) and long-term (6 and 12 weeks) exposure of EA.hy926 cells to PFOA.

Table 1. Average BMC lower confidence limit (BMCL, i.e. POD), BMC, and BMC upper confidence limit (BMCU) values for transcriptomics (T) data following short-term (48 h) and long-term (6 and 12 weeks) exposure of EA.hy926 cells to PFOA..

	BMCL (POD)	BMC	BMCU
T-48 h	6.3 µM	10.3 µM	36.8 µM
T-6 weeks	4.1 nM	6.8 nM	19.9 nM
T-12 weeks	22.1 nM	36.8 nM	134 nM



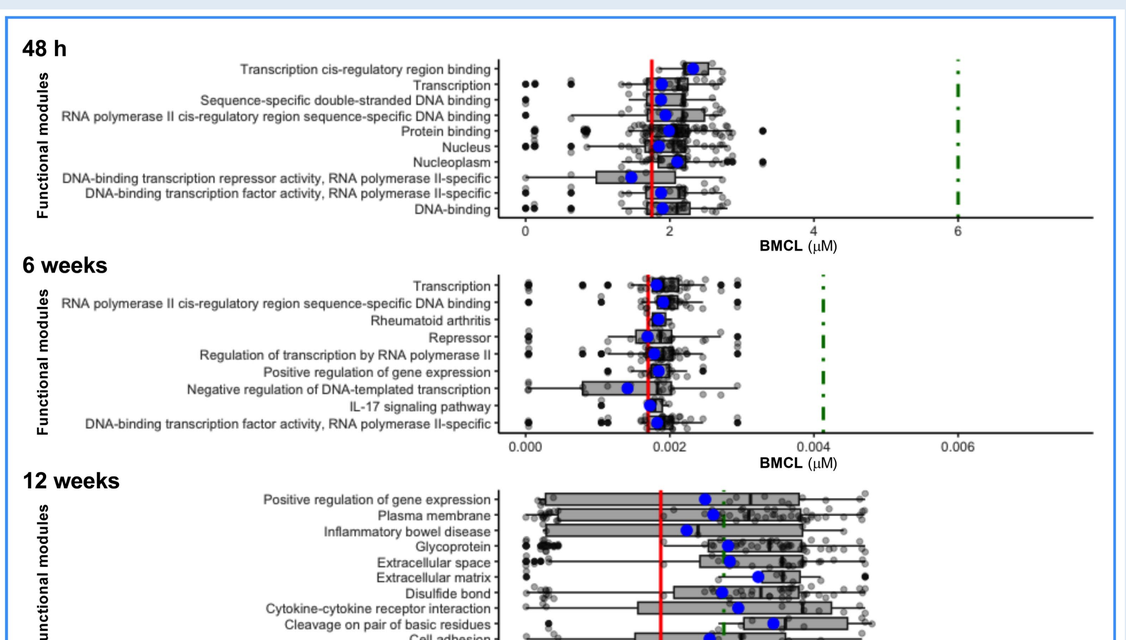


Figure 4. Density plots illustrating the distribution of benchmark concentration (BMC) values following short-term (48 h) and long-term (6 and 12 weeks) exposure of EA.hy926 cells to PFOA. For improved visualization, the smaller panel within the main panel shows a specific narrow range of BMC values.

Figure 5. Benchmark concentration lower confidence limit (BMCL) values of individual genes classified into functional modules. The grey box represents the range between the 1st and the 3rd quartiles, while the thick black line indicates the mean. The red vertical line marks the lowest 25th percentile BMCL, and the green dotted vertical line indicates the median BMCL value at each exposure time point.

Acknowledgements

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