Supplementary material

^{1}H NMR metabolomics of *Eisenia fetida* responses after sub-lethal exposure to perfluorooctanoic acid and perfluorooctane sulfonate

Brian P. Lankadurai,[A] *André J. Simpson*[A] *and Myrna J. Simpson*[A,B]

[A]Department of Chemistry, University of Toronto, 1265 Military Trail, Toronto, ON, M1C 1A4, Canada.

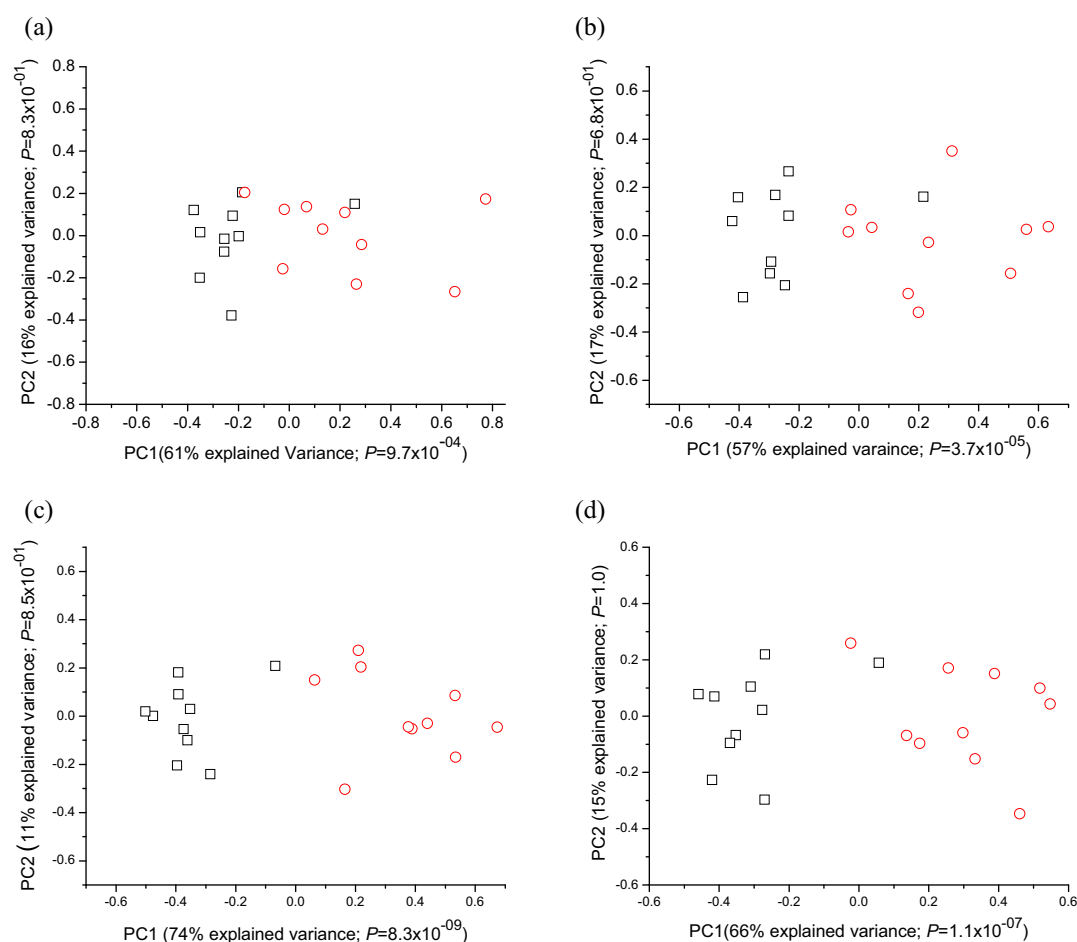[B]Corresponding author. Email: myrna.simpson@utoronto.ca

Fig. S1. PCA scores plots of PC1 (first PCA component) *v.* PC2 (second PCA component) for ^{1}H NMR spectra of the *E. fetida* tissue extracts showing the separation of control earthworms (□) from exposed earthworms (○) after PFOA exposures of (a) 6.25 µg cm^{-2}, (b) 12.5 µg cm^{-2}, (c) 25 µg cm^{-2} and (d) 50 µg cm^{-2}. The *P*-values were obtained from t-tests (two tailed, equal variances) that compared the scores of the control and exposed earthworms for each component.
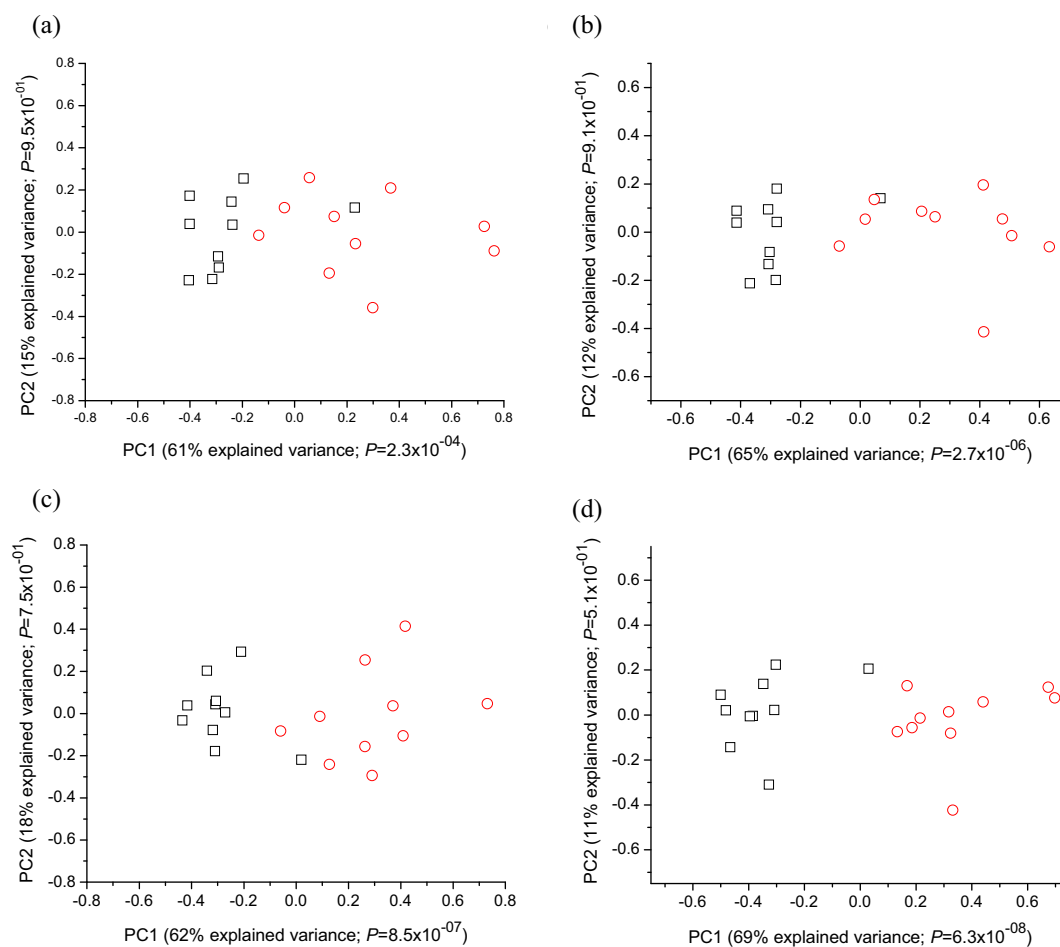
Fig. S2. PCA scores plots of PC1 (first PCA component) *v.* PC2 (second PCA component) for ^{1}H NMR spectra of the *E. fetida* tissue extracts showing the separation of control earthworms (□) from exposed earthworms (○) after PFOS exposures of (a) 3.125 µg cm^{-2}, (b) 6.25 µg cm^{-2}, (c) 12.5 µg cm^{-2} and (d) 25 µg cm^{-2}. The *P*-values were obtained from t-tests (two tailed, equal variances) that compared the scores of the control and exposed earthworms for each component.
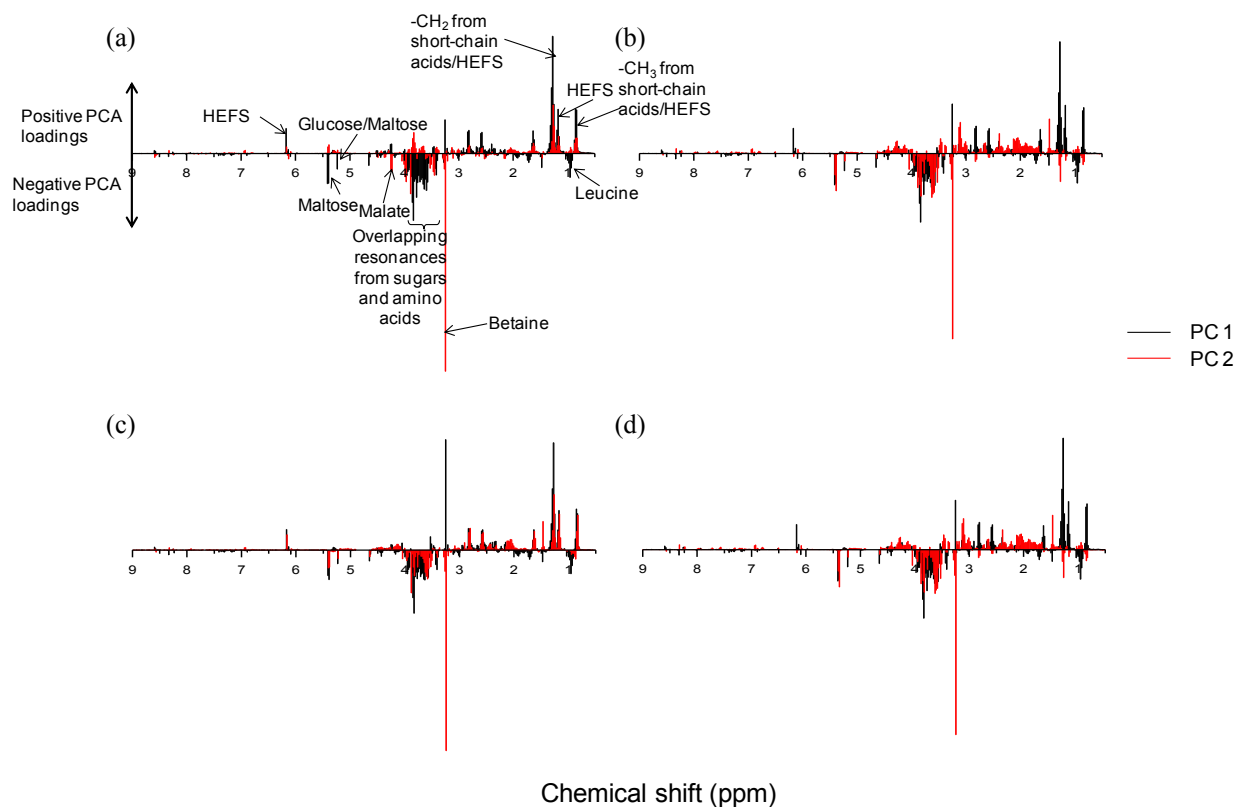
Chemical shift (ppm)

Fig. S3. Loadings plot for PC1 and PC2 showing the metabolites that were major contributors to the separation observed in the PCA scores plot of the *E. fetida* tissue extracts comparing the controls and PFOA exposed earthworms. The abscissa refers to the ^{1}H NMR chemical shifts (ppm). The loadings plots refer to PFOA exposures of (a) 6.25 µg cm^{-2}, (b) 12.5 µg cm^{-2}, (c) 25 µg cm^{-2} and (d) 50 µg cm^{-2}.
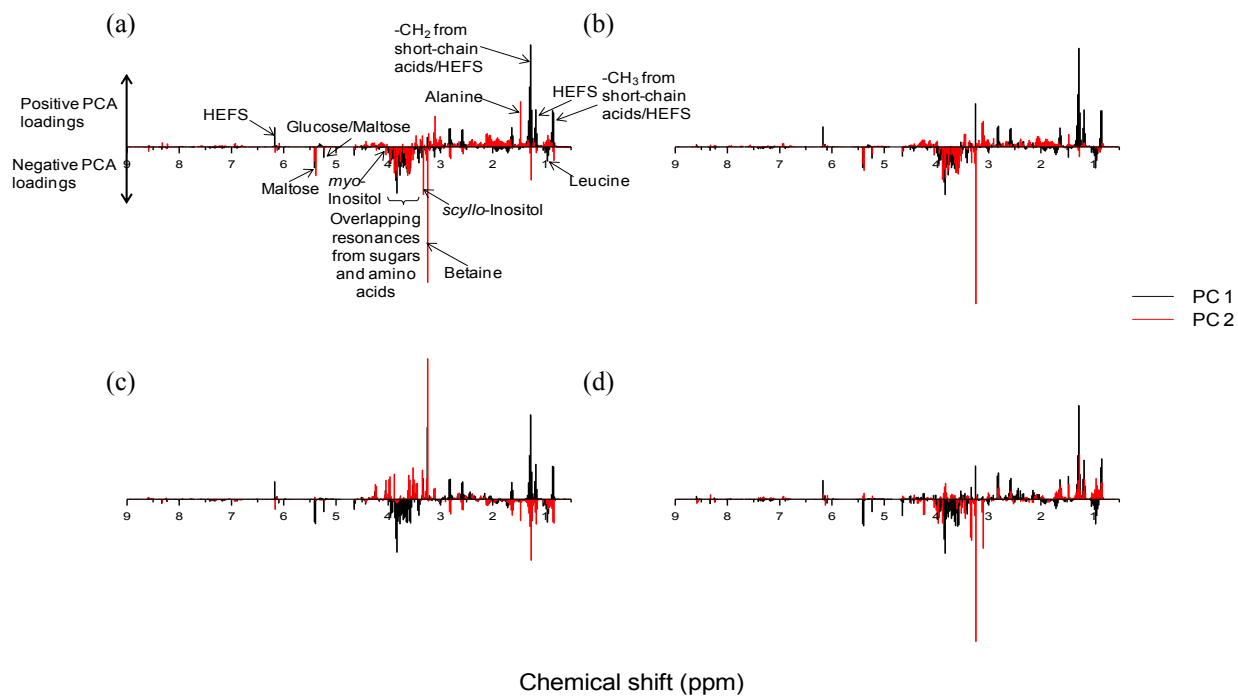
Fig. S4. Loadings plot for PC1 and PC2 showing the metabolites that were major contributors to the separation observed in the PCA scores plot of the *E. fetida* tissue extracts comparing the controls and PFOS exposed earthworms. The abscissa refers to the ^{1}H NMR chemical shifts (ppm). The loadings plots refer to PFOS exposures of (a) 3.125 µg cm^{-2}, (b) 6.25 µg cm^{-2}, (c) 12.5 µg cm^{-2} and (d) 25 µg cm^{-2}.
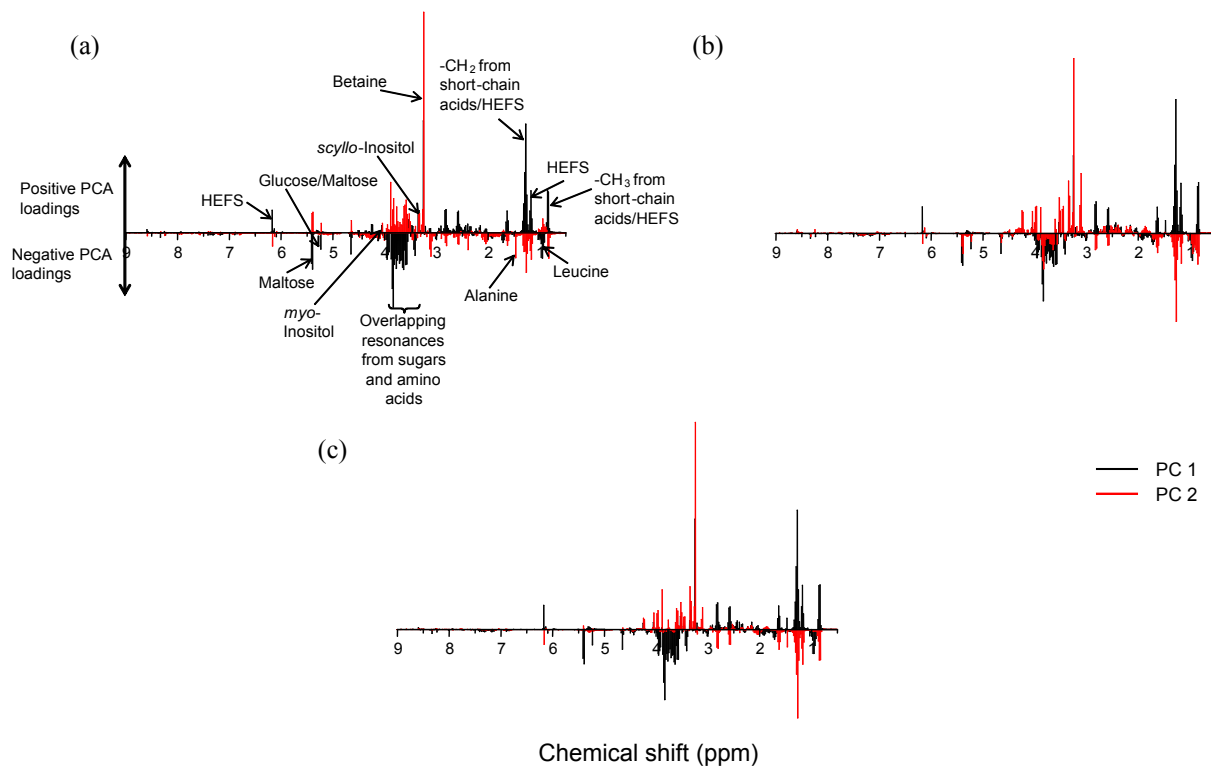
Fig. S5. Loadings plot for PC1 and PC2 showing the metabolites that were major contributors to the separation observed in the average PCA scores plots of the *E. fetida* tissue extracts comparing the controls and PFOA and PFOS exposed earthworms. The abscissa refers to the ^{1}H NMR chemical shifts (ppm). The loadings plots refer to PFOS exposures of (A) PFOA exposure, (B) PFOS exposure, and (C) PFOA and PFOS exposure.
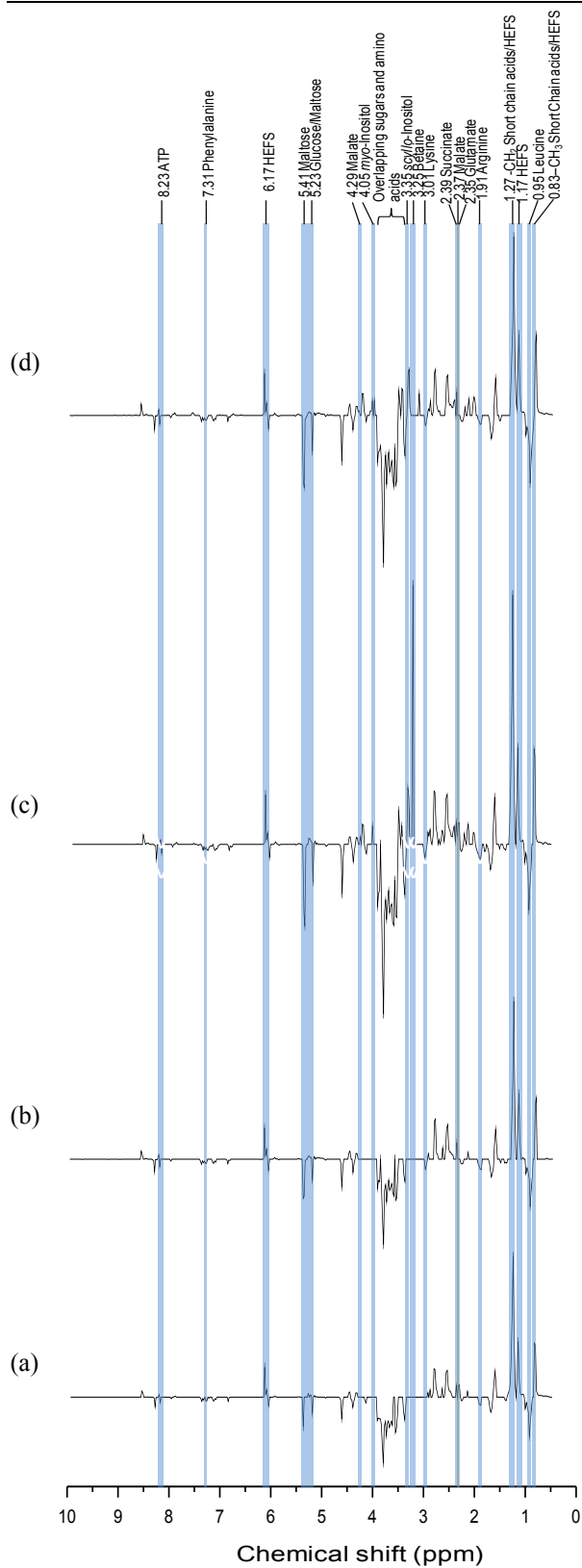
Fig. S6. T-test filtered ^{1}H NMR difference spectra of the *E. fetida* tissue extracts obtained by subtracting the mean buckets of the control earthworms from the mean buckets for the PFOA exposed earthworms and retaining the buckets that were statistically different from the controls at $\alpha = 0.05$. The t-test filtered ^{1}H NMR difference spectra are shown after PFOA exposure of (a) 6.25 µg cm^{-2}, (b) 12.5 µg cm^{-2}, (c) 25 µg cm^{-2} and (d) 50 µg cm^{-2}.
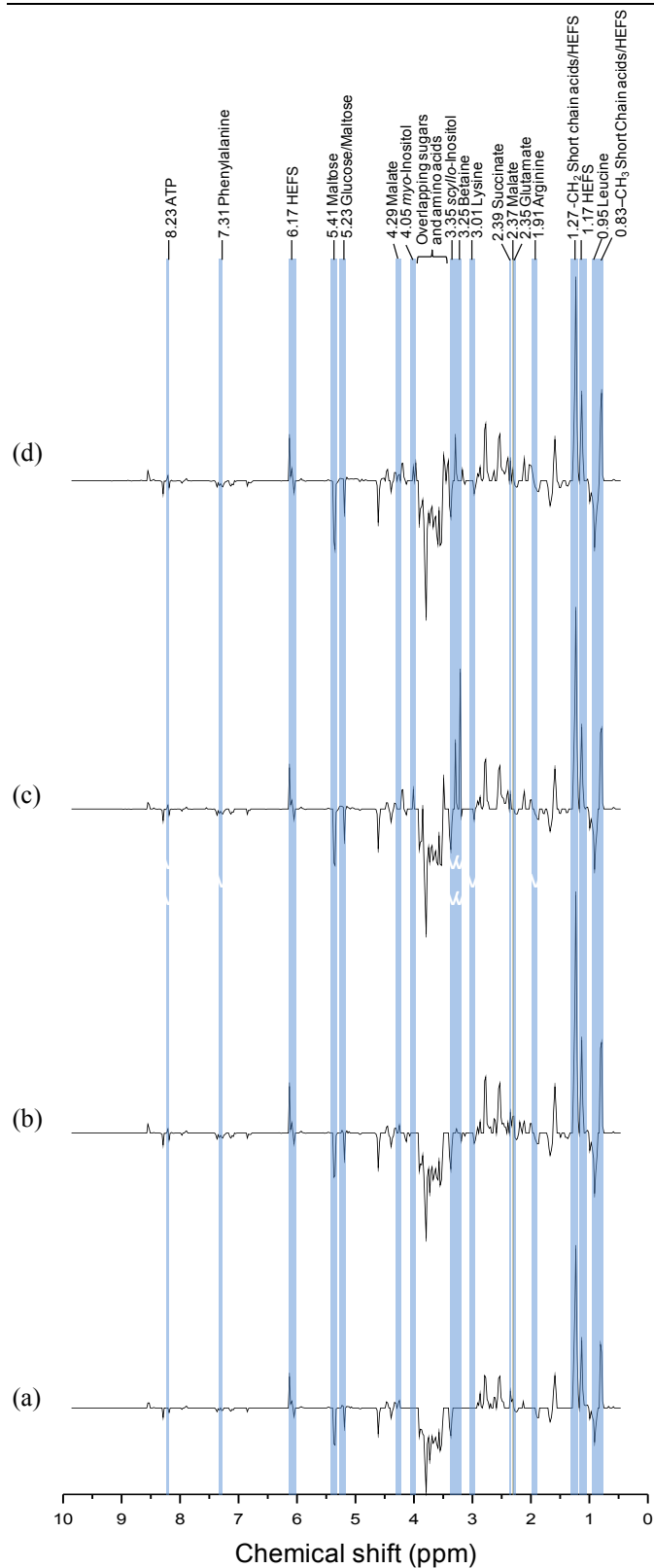
Fig. S7. T-test filtered ^{1}H NMR difference spectra of the *E. fetida* tissue extracts obtained by subtracting the mean buckets of the control earthworms from the mean buckets for the PFOS exposed earthworms and retaining the buckets that were statistically different from the controls at α = 0.05. The t-test filtered ^{1}H NMR difference spectra are shown after PFOS exposure of (a) 3.125 µg cm^{-2}, (b) 6.25 µg cm^{-2}, (c) 12.5 µg cm^{-2} and (d) 25 µg cm^{-2}.