Environ Health Perspect

DOI: 10.1289/EHP5843

Supplemental Material

Evaluation of Developmental Toxicity, Developmental Neurotoxicity, and Tissue Dose in Zebrafish Exposed to GenX and Other PFAS

Shaza Gaballah, Adam Swank, Jon R. Sobus, Xia Meng Howey, Judith Schmid, Tara Catron, James McCord, Erin Hines, Mark Strynar, and Tamara Tal

Table of Contents

Figure S3. Changes in movement of zebrafish exposed to ADONA, PFOA, PFESA1, PFHxA, PFHxS, or PFOS. Zebrafish were semi-statically exposed to 4.4-80.0 μM ADONA, PFOA, PFESA1, PFHxA or PFHxS, 0.2-3.1 μM PFOS, or 0.4% DMSO daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. Changes in movement across the light (A, C, E, G, I, K) or dark (B, D, F, H, J, L) period are shown. For all chemicals except PFESA1, 14-23 larvae were tested per chemical concentration and the same DMSO control larvae (n=394) were used. PFESA1 was tested separately (n=35-40 per chemical per concentration; 339 DMSO control larvae were evaluated). Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

Figure S4. Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point for a given chemical corresponds to a different test concentration.

Figure S5. GenX Free Acid Stock Degradation. 20 mM GenX Free Acid stocks were prepared in 20 ml glass vials. Fresh solution was retained in the glass vial and a subset was added to a plastic microcentrifuge tube. Samples from plastic and glass containers were collected and analyzed immediately afterwards. Stocks were stored at room temperature in the dark. Samples were collected and analyzed 24 hr later.

Figure S6. Changes in movement of zebrafish exposed to GenX Free Acid diluted in DI water. Zebrafish were semi-statically exposed to 4.4-80.0 μM GenX Free Acid in DI water as a vehicle control daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. (A) Changes in movement across the light period or (B) dark period are shown. 17-21 larvae were tested per chemical concentration and 161 control larvae were used. Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

Figure S7. Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point corresponds to a different test concentration.

Figure S8. Changes in movement of zebrafish exposed to aliphatic sulfonic acid PFAS. Zebrafish were semi-statically exposed to 5.5-100.0 μM PFBS, 3.1-56.0 μM PFPeS, 3.1-31.4 μM PFHxS, 1.7-9.8 μM PFHpS, 0.5-1.7 μM PFOS, or 0.4% DMSO as a vehicle control daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. (A, C, E, G, I) Changes in movement across the light period or (B, D, F, H, J) dark period are shown. For all chemicals except PFPeS, 14-25 larvae were tested per chemical concentration and the same DMSO control larvae (n=327) were used. PFPeS was tested separately (n=21-22 per chemical per concentration; 186 DMSO control larvae were evaluated). Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

Figure S9. Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point for a given chemical corresponds to a different test concentration.

Additional File- Excel Document

Supplemental Figures

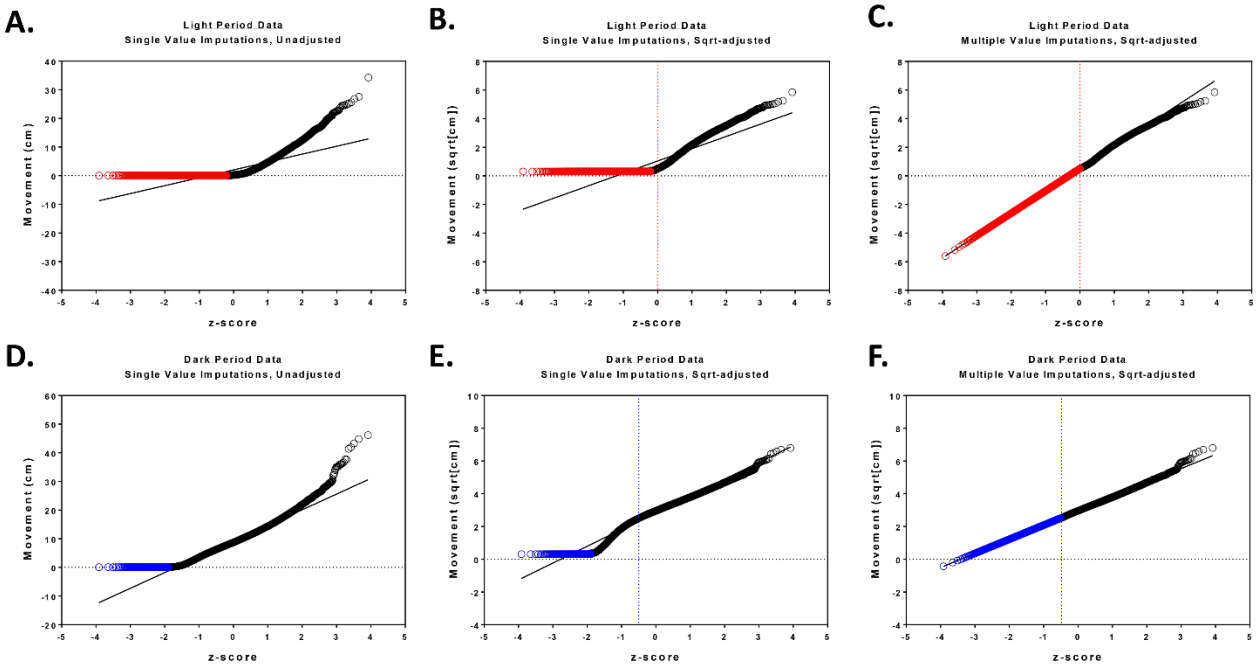


Figure S1: Transformation of movement data in the developmental neurotoxicity assay. Zebrafish were semi-statically exposed to 4.4-80.0 µM ADONA, PFOA, PFESA1, PFHxA or PFHxS, 0.2-3.1 µM PFOS, or DMSO as a vehicle control (0.4%) daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. QQ plots are shown for distance moved during each 20 min period (light or dark) with (A, D) unadjusted single value imputations, (B, E) square-root adjusted single value imputations, and (C, F) square-root adjusted data with multiple value imputations. Data points that were below the limit of detection (LOD) (0.135 cm) in the light (red dots) or dark (blue dots) periods are indicated. For all chemicals except PFESA1, 14-23 larvae were tested per chemical concentration and the same DMSO control larvae (n=394) were used. PFESA1 was tested separately (n=35-40 per chemical per concentration; 339 DMSO control larvae were evaluated).

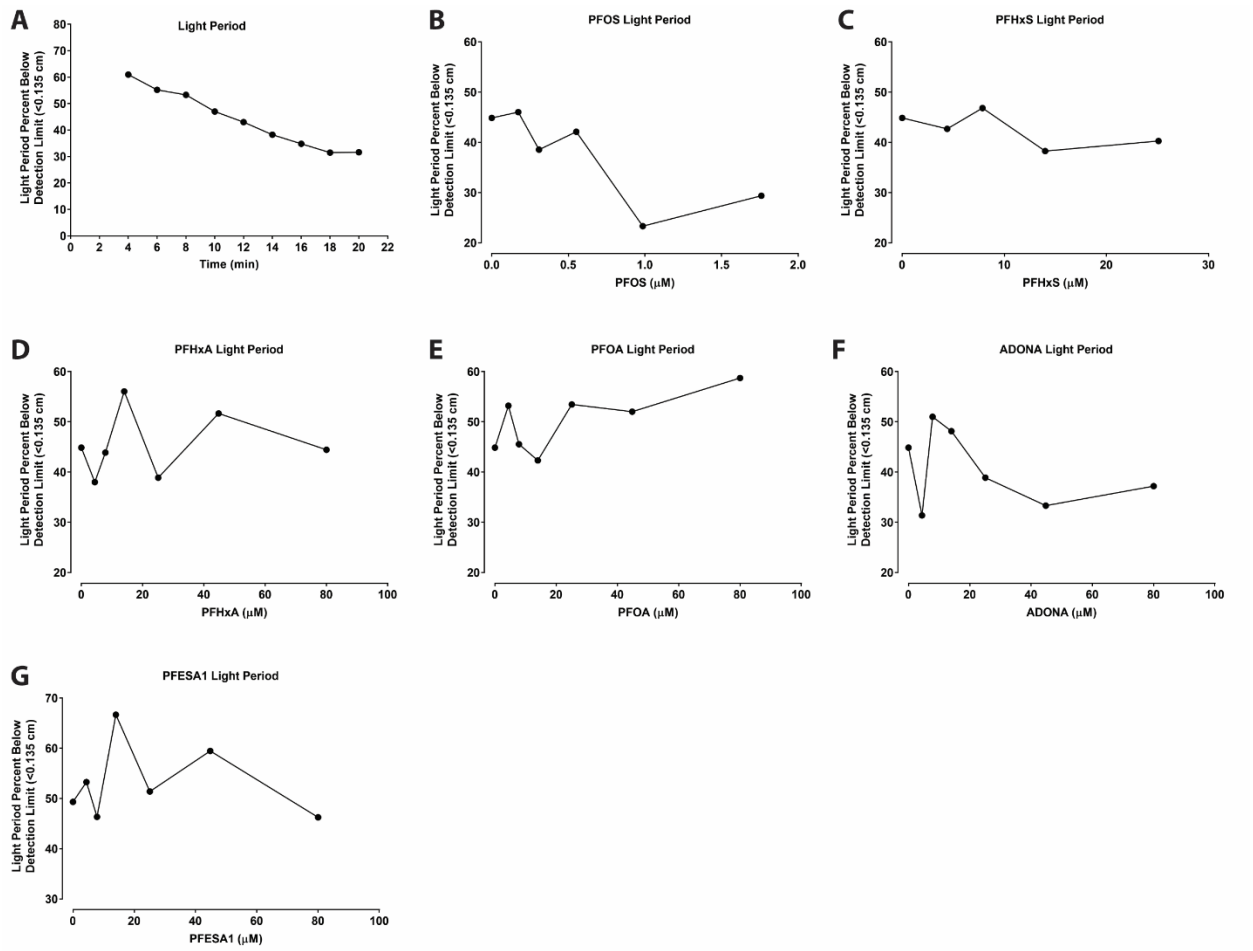
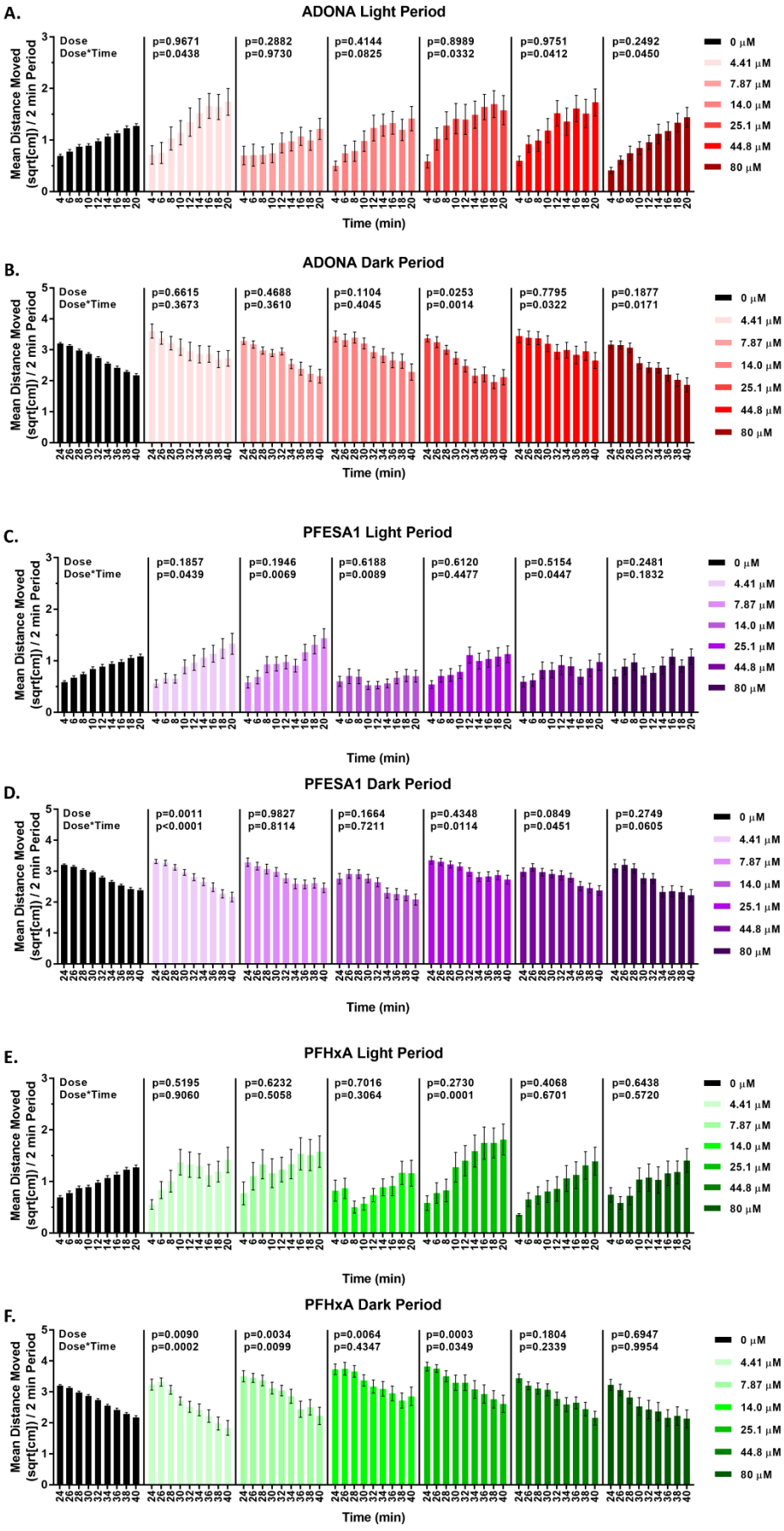
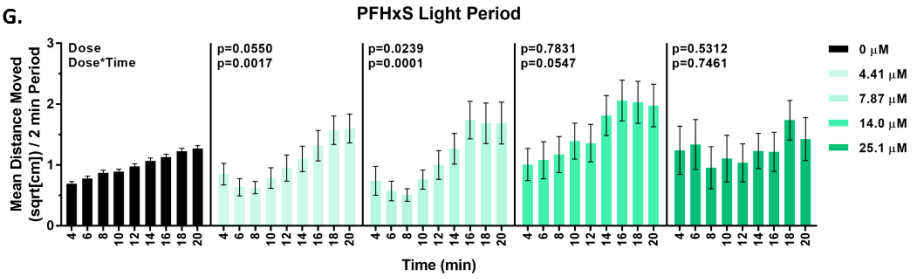


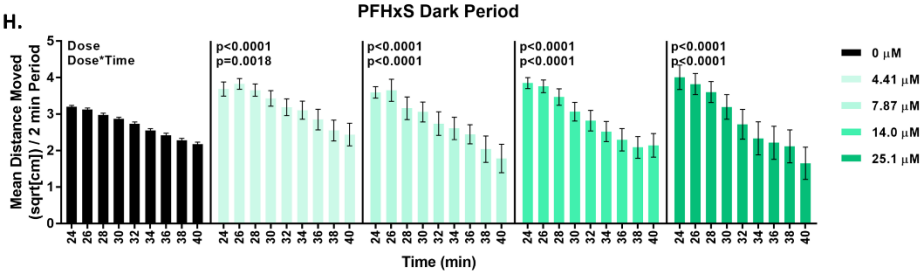
Figure S2: Relationship between LOD values and time or chemical concentration. Zebrafish were semi-statically exposed to 4.4-80.0 µM ADONA, PFOA, PFESA1, PFHxA or PFHxS, 0.2-3.1 µM PFOS, or DMSO as a vehicle control (0.4%) daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing, square-root adjustment, and multiple value imputations. The number of data points that were <LOD (0.135 cm) in the light period were plotted against (A) time or chemical concentration for (B) PFOS, (C) PFHxS, (D) PFHxA, (E) PFOA, (F) ADONA, or (G) PFESA1. An inverse trend between time and measurements <LOD was observed. A more modest inverse relationship between chemical concentration and measurements <LOD was also observed for chemicals that caused light-phase hyperactivity (i.e. PFOS and PFHxS). For all chemicals except PFESA1, 14-23 larvae were tested per chemical concentration and the same DMSO control larvae (n=394) were used. PFESA1 was tested separately (n=35-40 per chemical per concentration; 339 DMSO control larvae were evaluated).


A.
ADONA Light Period
Dose
Dose*Time
p=0.9671
p=0.0438
p=0.2882
p=0.9730
p=0.4144
p=0.0825
p=0.8989
p=0.0332
p=0.9751
p=0.0412
p=0.2492
p=0.0450
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM
B.
ADONA Dark Period
Dose
Dose*Time
p=0.6615
p=0.3673
p=0.4688
p=0.3610
p=0.1104
p=0.4045
p=0.0253
p=0.0014
p=0.7795
p=0.0322
p=0.1877
p=0.0171
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM
C.
PFESA1 Light Period
Dose
Dose*Time
p=0.1857
p=0.0439
p=0.1946
p=0.0069
p=0.6188
p=0.0089
p=0.6120
p=0.4477
p=0.5154
p=0.0447
p=0.2481
p=0.1832
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM
D.
PFESA1 Dark Period
Dose
Dose*Time
p=0.0011
p<0.0001
p=0.9827
p=0.8114
p=0.1664
p=0.7211
p=0.4348
p=0.0114
p=0.0849
p=0.0451
p=0.2749
p=0.0605
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM
E.
PFHxA Light Period
Dose
Dose*Time
p=0.5195
p=0.9060
p=0.6232
p=0.5058
p=0.7016
p=0.3064
p=0.2730
p=0.0001
p=0.4068
p=0.6701
p=0.6438
p=0.5720
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM
F.
PFHxA Dark Period
Dose
Dose*Time
p=0.0090
p=0.0002
p=0.0034
p=0.0099
p=0.0064
p=0.4347
p=0.0003
p=0.0349
p=0.1804
p=0.2339
p=0.6947
p=0.9954
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
0 µM
4.41 µM
7.87 µM
14.0 µM
25.1 µM
44.8 µM
80 µM

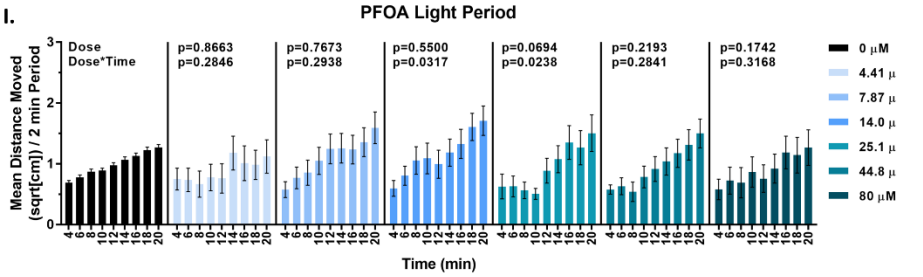
G.


PFHxS Light Period
Dose
Dose*Time
p=0.0550
p=0.0017
p=0.0239
p=0.0001
p=0.7831
p=0.0547
p=0.5312
p=0.7461
0 μM
4.41 μM
7.87 μM
14.0 μM
25.1 μM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

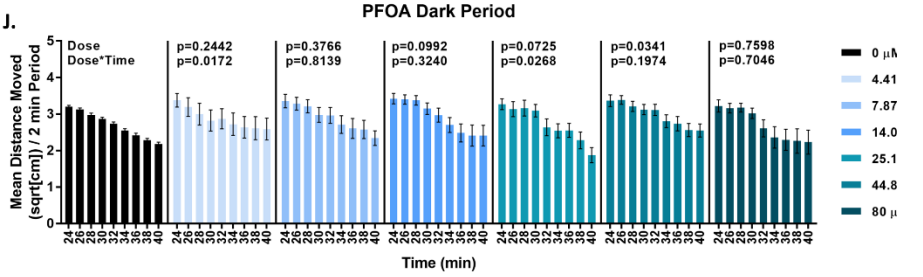
H.


PFHxS Dark Period
Dose
Dose*Time
p<0.0001
p=0.0018
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
0 μM
4.41 μM
7.87 μM
14.0 μM
25.1 μM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

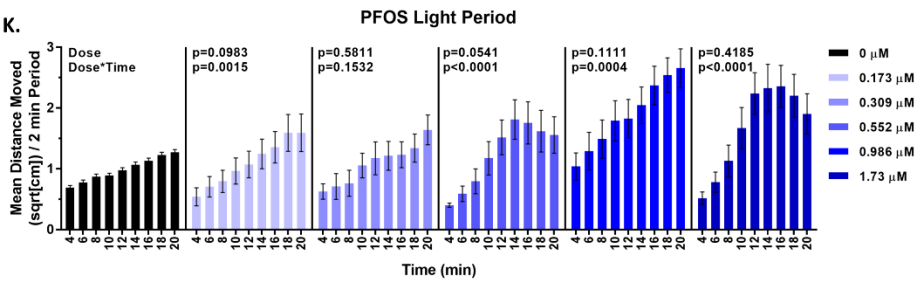
I.


PFOA Light Period
Dose
Dose*Time
p=0.8663
p=0.2846
p=0.7673
p=0.2938
p=0.5500
p=0.0317
p=0.0694
p=0.0238
p=0.2193
p=0.2841
p=0.1742
p=0.3168
0 μM
4.41 μ
7.87 μ
14.0 μ
25.1 μ
44.8 μ
80 μM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

J.


PFOA Dark Period
Dose
Dose*Time
p=0.2442
p=0.0172
p=0.3766
p=0.8139
p=0.0992
p=0.3240
p=0.0725
p=0.0268
p=0.0341
p=0.1974
p=0.7598
p=0.7046
0 μM
4.41
7.87
14.0
25.1
44.8
80 μ
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

K.


PFOS Light Period
Dose
Dose*Time
p=0.0983
p=0.0015
p=0.5811
p=0.1532
p=0.0541
p<0.0001
p=0.1111
p=0.0004
p=0.4185
p<0.0001
0 μM
0.173 μM
0.309 μM
0.552 μM
0.986 μM
1.73 μM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

L.

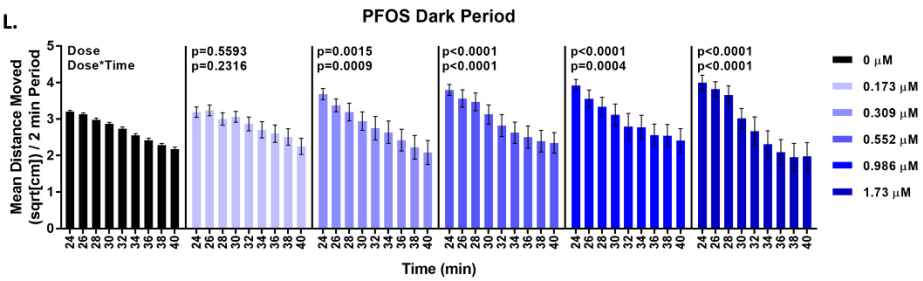

PFOS Dark Period
Dose
Dose*Time
p=0.5593
p=0.2316
p=0.0015
p=0.0009
p<0.0001
p<0.0001
p<0.0001
p=0.0004
p<0.0001
p<0.0001
0 μM
0.173 μM
0.309 μM
0.552 μM
0.986 μM
1.73 μM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

Figure S3: Changes in movement of zebrafish exposed to ADONA, PFOA, PFESA1, PFHxA, PFHxS, or PFOS. Zebrafish were semi-statically exposed to 4.4-80.0 µM ADONA, PFOA, PFESA1, PFHxA or PFHxS, 0.2-3.1 µM PFOS, or 0.4% DMSO daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. Changes in movement across the light (A, C, E, G, I, K) or dark (B, D, F, H, J, L) period are shown. For all chemicals except PFESA1, 14-23 larvae were tested per chemical concentration and the same DMSO control larvae (n=394) were used. PFESA1 was tested separately (n=35-40 per chemical per concentration; 339 DMSO control larvae were evaluated). Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

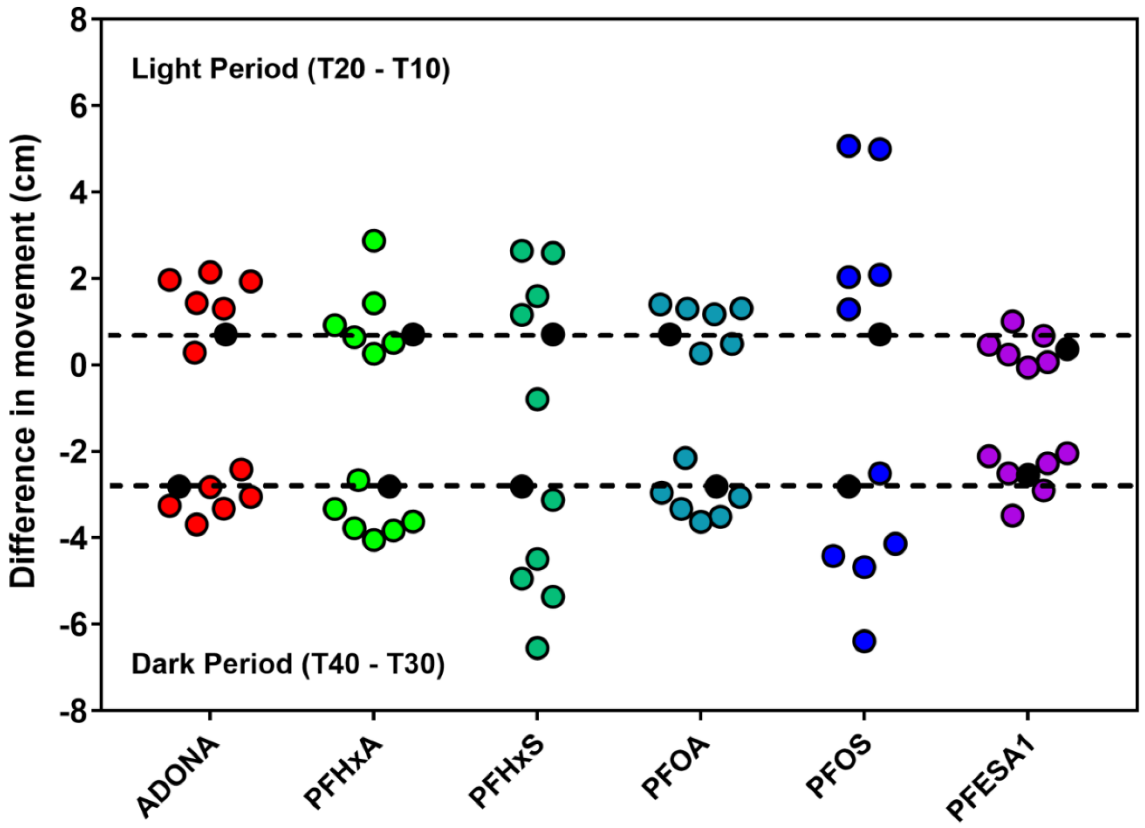


Figure S4: Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point for a given chemical corresponds to a different test concentration.

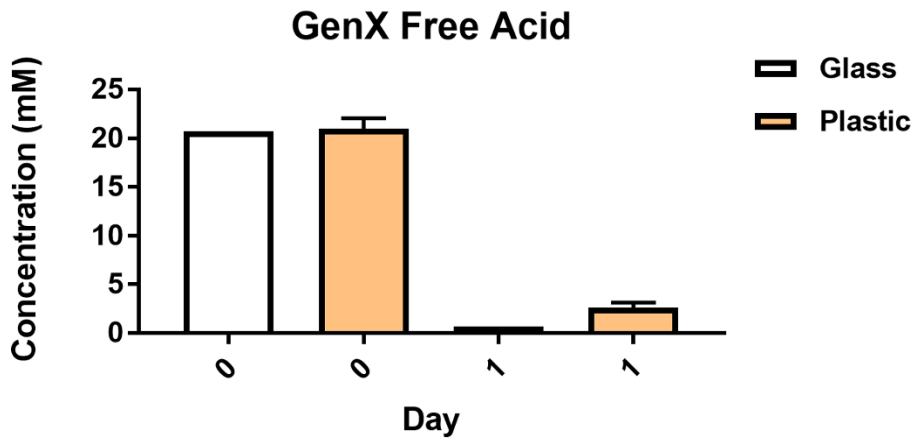


Figure S5: GenX Free Acid Stock Degradation. 20 mM GenX Free Acid stocks were prepared in 20 ml glass vials. Fresh solution was retained in the glass vial and a subset was added to a plastic microcentrifuge tube. Samples from plastic and glass containers were collected and analyzed immediately afterwards. Stocks were stored at room temperature in the dark. Samples were collected and analyzed 24 hr later.

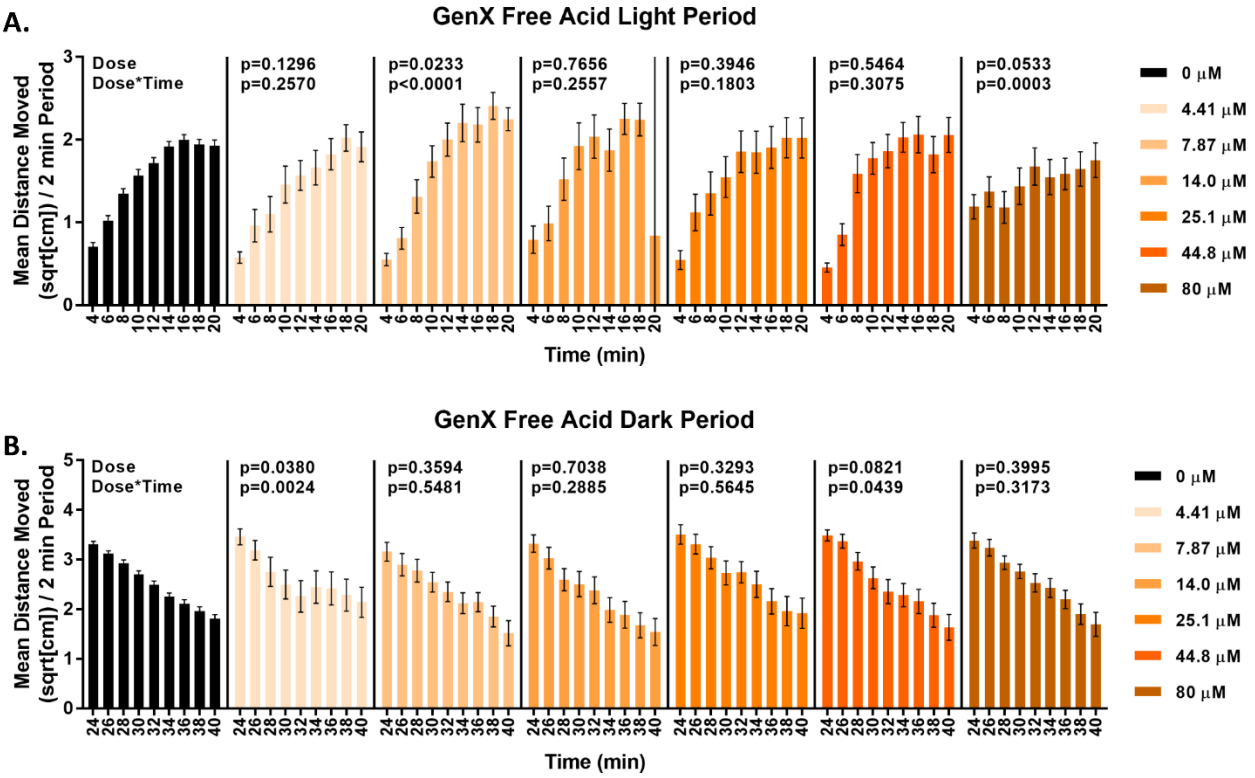


Figure S6: Changes in movement of zebrafish exposed to GenX Free Acid diluted in DI water. Zebrafish were semi-statically exposed to 4.4-80.0 µM GenX Free Acid in DI water as a vehicle control daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. (A) Changes in movement across the light period or (B) dark period are shown. 17-21 larvae were tested per chemical concentration and 161 control larvae were used. Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

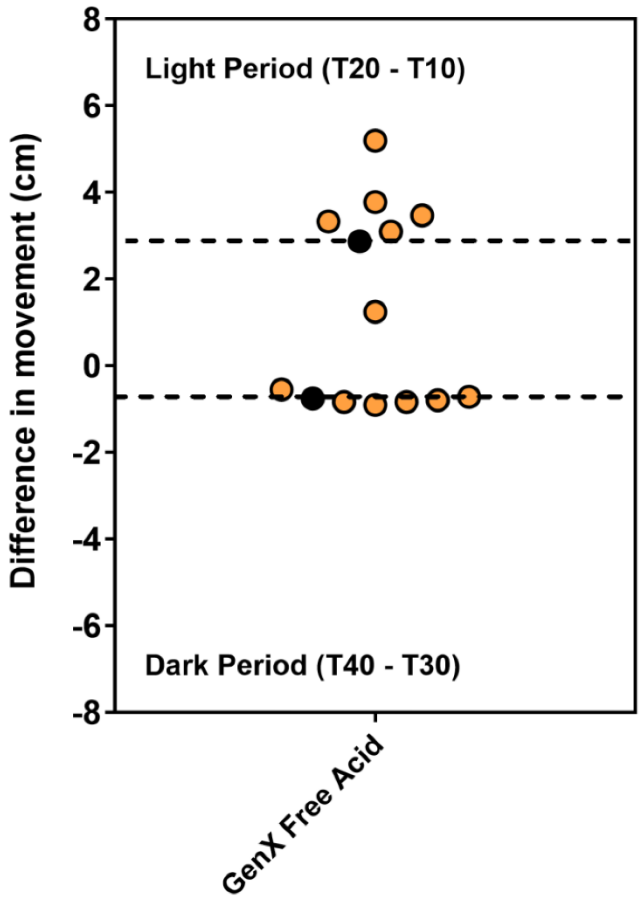
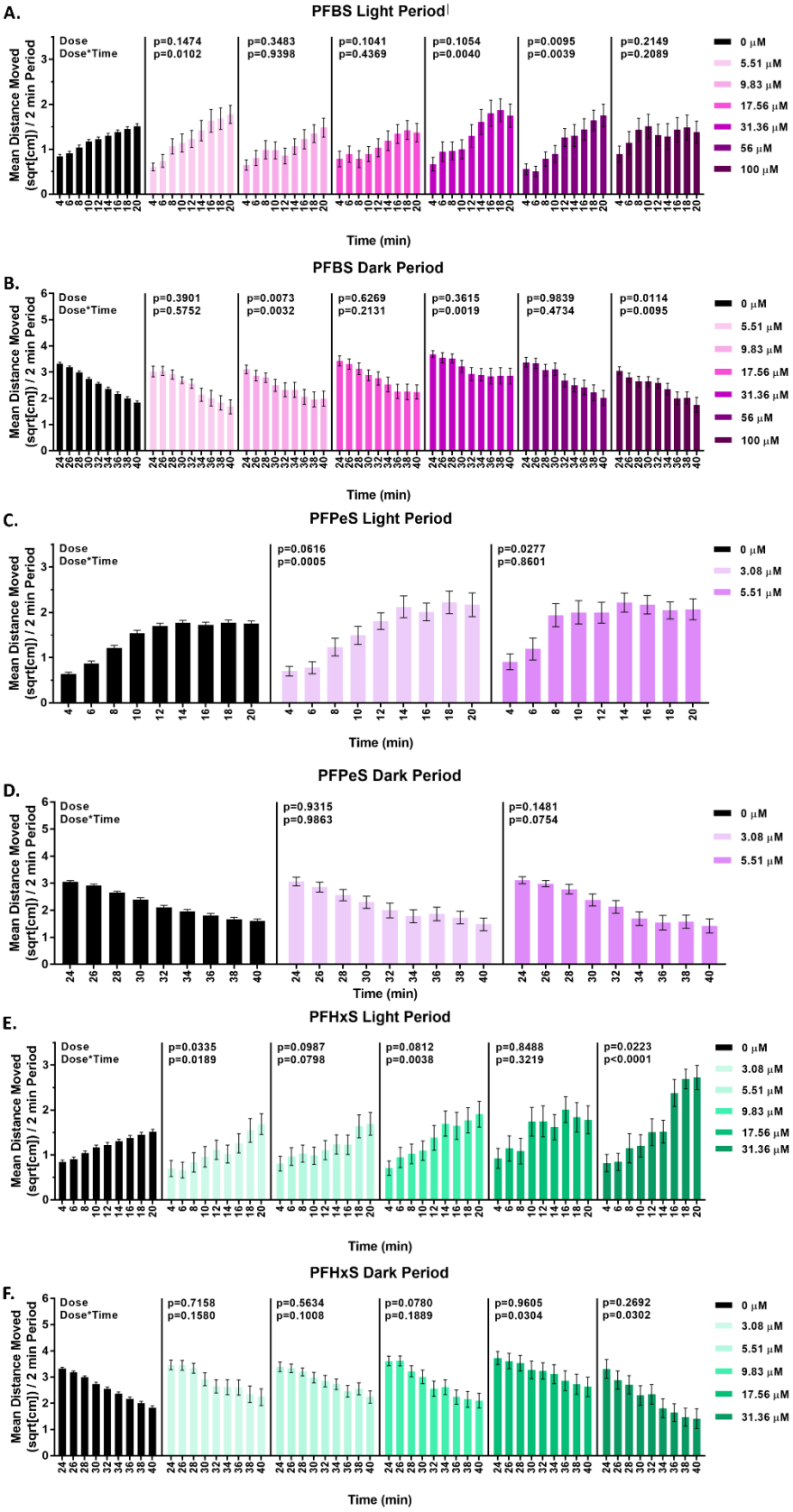


Figure S7: Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point corresponds to a different test concentration.


A.
PFBS Light Period
Dose
Dose*Time
p=0.1474
p=0.0102
p=0.3483
p=0.9398
p=0.1041
p=0.4369
p=0.1054
p=0.0040
p=0.0095
p=0.0039
p=0.2149
p=0.2089
0 µM
5.51 µM
9.83 µM
17.56 µM
31.36 µM
56 µM
100 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
B.
PFBS Dark Period
Dose
Dose*Time
p=0.3901
p=0.5752
p=0.0073
p=0.0032
p=0.6269
p=0.2131
p=0.3615
p=0.0019
p=0.9839
p=0.4734
p=0.0114
p=0.0095
0 µM
5.51 µM
9.83 µM
17.56 µM
31.36 µM
56 µM
100 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
C.
PFPeS Light Period
Dose
Dose*Time
p=0.0616
p=0.0005
p=0.0277
p=0.8601
0 µM
3.08 µM
5.51 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
D.
PFPeS Dark Period
Dose
Dose*Time
p=0.9315
p=0.9863
p=0.1481
p=0.0754
0 µM
3.08 µM
5.51 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
E.
PFHxS Light Period
Dose
Dose*Time
p=0.0335
p=0.0189
p=0.0987
p=0.0798
p=0.0812
p=0.0038
p=0.8488
p=0.3219
p=0.0223
p<0.0001
0 µM
3.08 µM
5.51 µM
9.83 µM
17.56 µM
31.36 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)
F.
PFHxS Dark Period
Dose
Dose*Time
p=0.7158
p=0.1580
p=0.5634
p=0.1008
p=0.0780
p=0.1889
p=0.9605
p=0.0304
p=0.2692
p=0.0302
0 µM
3.08 µM
5.51 µM
9.83 µM
17.56 µM
31.36 µM
Mean Distance Moved (sqrt[cm]) / 2 min Period
Time (min)

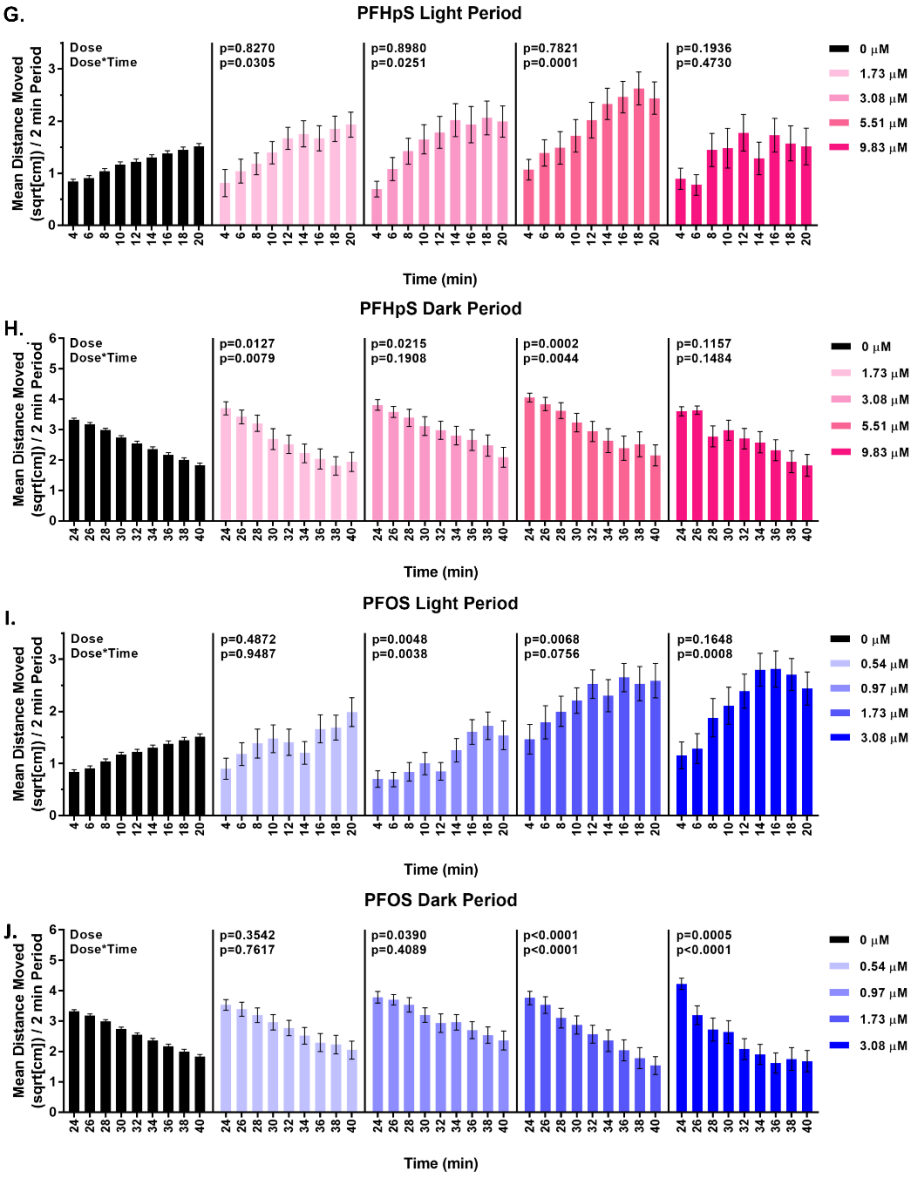


Figure S8: Changes in movement of zebrafish exposed to aliphatic sulfonic acid PFAS. Zebrafish were semi-statically exposed to 5.5-100.0 µM PFBS, 3.1-56.0 µM PFPeS, 3.1-31.4 µM PFHxS, 1.7-9.8 µM PFHpS, 0.5-1.7 µM PFOS, or 0.4% DMSO as a vehicle control daily from 0-5 dpf. At 6 dpf, larvae were assessed for developmental toxicity. Morphologically normal larvae with inflated swim bladders were subjected to behavioral testing. (A, C, E, G, I) Changes in movement across the light period or (B, D, F, H, J) dark period are shown. For all chemicals except PFPeS, 14-25 larvae were tested per chemical concentration and the same DMSO control larvae (n=327) were used. PFPeS was tested separately (n=21-22 per chemical per concentration; 186 DMSO control larvae were evaluated). Data were square-root adjusted with multiple value imputations for non-detects. A mixed model was used to assess global dose-related effects on mobility, and each group was compared to the control group to determine significance ($p<0.05$).

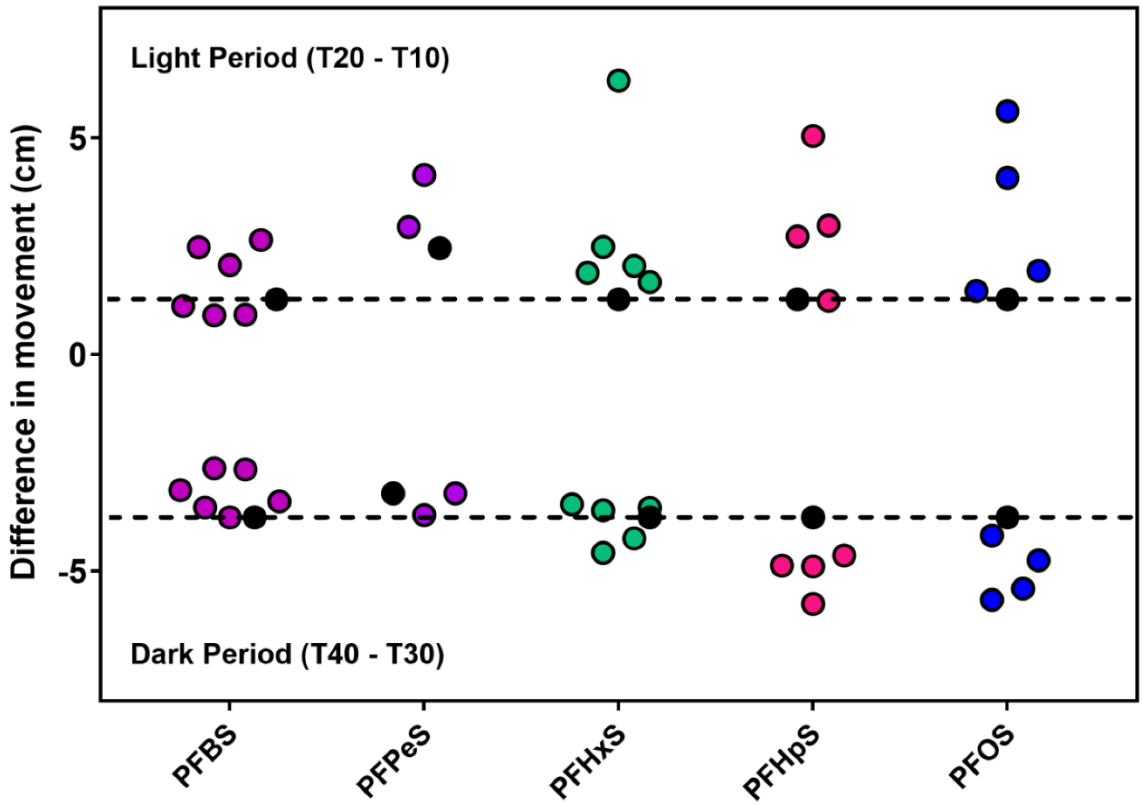


Figure S9: Estimated differences in movement of zebrafish based on mixed model predictions for light and dark period data. Estimates for light period data were based differences in movement between time period T20 and T10. Estimates for dark period data were based on differences in movement between time period T40 and T30. Each point for a given chemical corresponds to a different test concentration.