

## Supplementary Data

### **Advanced morphological-behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants**

Pamela D. Noyes\*, Derik E. Haggard\*, Greg D. Gonnerman\*, Robert L. Tanguay\*†

\* Department of Environmental & Molecular Toxicology, Environmental Health Sciences Center, Oregon State University, Corvallis, OR 97331

† Corresponding author: Phone: 541-737-6514; Email: robert.tanguay@oregonstate.edu.

1. **Sample Sizes**  
**Embryonic Photomotor Responses at 24 hpf**

| Abbr                            | Chemical                                      | Sample size by test concentration (µM) |              |              |              |              |       |      |     |    |
|---------------------------------|-----------------------------------------------|----------------------------------------|--------------|--------------|--------------|--------------|-------|------|-----|----|
|                                 |                                               | 0                                      | 6.40<br>E-06 | 6.40<br>E-05 | 6.40<br>E-04 | 6.40<br>E-03 | 0.064 | 0.64 | 6.4 | 64 |
| Chlorinated Phosphate Ester FRs |                                               |                                        |              |              |              |              |       |      |     |    |
| BDP                             | bisphenol A bis-(diphenyl phosphate)          | 31                                     |              |              | 29           | 30           | 28    | 30   | 31  |    |
| BPDP                            | <i>t</i> -Butylphenyl diphenyl phosphate      | 31                                     |              |              |              | 29           | 30    | 28   | 29  | 21 |
| EHDP                            | 2-Ethylhexyl diphenyl phosphate               | 29                                     |              |              |              | 27           | 28    | 32   | 30  | 26 |
| IDDP                            | Isodecyl diphenyl phosphate                   | 30                                     |              |              |              | 32           | 28    | 29   | 29  | 23 |
| IPP-1                           | Isopropylated triphenyl phosphate - 1         | 31                                     |              |              |              | 31           | 31    | 32   | 31  | 26 |
| IPP-2                           | Isopropylated triphenyl phosphate - 2         | 31                                     |              |              |              | 28           | 30    | 29   | 28  | 29 |
| IPP-3                           | Isopropylated triphenyl phosphate - 3         | 30                                     |              |              |              | 32           | 27    | 30   | 27  | 17 |
| mITP                            | Mono isopropylated triaryl phosphate          | 31                                     |              | 32           | 32           | 30           | 30    | 29   |     |    |
| RDP                             | Resorcinol A bis-(diphenyl phosphate)         | 30                                     |              |              | 29           | 31           | 32    | 27   | 29  |    |
| TBP                             | Tributyl phosphate                            | 30                                     |              |              | 27           | 30           | 28    | 31   | 31  |    |
| TBEP                            | Tris (2-butoxyethyl) phosphate                | 32                                     |              |              | 32           | 32           | 31    | 30   | 32  |    |
| TCP                             | Tricresyl phosphate                           | 28                                     |              |              |              | 24           | 25    | 24   | 27  | 17 |
| o-TCP                           | Tri- <i>o</i> -cresyl phosphate               | 31                                     |              |              |              | 29           | 30    | 30   | 30  | 29 |
| TEHP                            | Tris (2-ethylhexyl) phosphate                 | 31                                     |              |              | 32           | 31           | 31    | 30   | 31  |    |
| TPP                             | Triphenyl phosphate                           | 32                                     |              |              |              | 28           | 28    | 26   | 30  | 29 |
| DPP*                            | Diphenyl phosphate                            | 31                                     |              |              | 31           | 30           | 32    | 31   | 32  |    |
| Chlorinated Phosphate Ester FRs |                                               |                                        |              |              |              |              |       |      |     |    |
| BCPCP*                          | Bis (2-chloropropyl) 1-carboxyethyl phosphate | 31                                     |              |              |              | 32           | 28    | 30   | 29  | 30 |
| BCPP*                           | Bis(1,3-dichloro-2-propyl)phosphate           | 32                                     |              |              |              | 32           | 30    | 31   | 31  | 31 |
| BDCPP*                          | Bis (2-chloropropyl) phosphate                | 32                                     |              |              |              | 24           | 30    | 26   | 30  | 29 |
| Dechlorane                      | Dechlorane Plus                               | 30                                     |              |              | 29           | 27           | 29    | 30   | 31  |    |
| MCPP*                           | Mono (2-chloropropyl) phosphate               | 31                                     |              |              |              | 31           | 31    | 30   | 31  | 32 |
| TCEP                            | Tris (1-chloropropyl) phosphate               | 31                                     |              |              |              | 31           | 32    | 29   | 31  | 31 |
| TCPP                            | Tris (2-chloroethyl) phosphate                | 30                                     |              |              |              | 32           | 30    | 32   | 31  | 29 |
| TDCPP                           | Tris (1,3-dichloro-2-propyl) phosphate        | 28                                     |              |              |              | 31           | 30    | 31   | 31  | 16 |
| PBDE FRs                        |                                               |                                        |              |              |              |              |       |      |     |    |
| DE-71                           | PentaBDE commercial mixture                   | 29                                     |              |              |              | 31           | 24    | 29   | 28  | 26 |
| DE-79                           | OctaBDE commercial mixture                    | 30                                     |              |              |              | 30           | 30    | 29   | 31  | 30 |
| BDE-3                           | <i>p</i> -Bromodiphenyl ether                 | 30                                     |              |              |              | 31           | 30    | 30   | 32  | 30 |
| BDE-15                          | <i>p,p</i> -Dibromodiphenyl ether             | 28                                     |              |              |              | 30           | 29    | 25   | 29  | 29 |
| BDE-47                          | 2,2',4,4'-Tetrabromodiphenyl ether            | 31                                     |              |              |              | 28           | 26    | 28   | 28  | 28 |
| BDE-99                          | 2,2',4,4',5-Pentabromodiphenyl ether          | 30                                     |              |              |              | 30           | 30    | 30   | 28  | 31 |
| BDE-100                         | 2,2',4,4',6-Pentabromodiphenyl ether          | 29                                     | 26           | 30           | 29           | 32           |       |      |     |    |
| BDE-153                         | 2,2',4,4',5,5'-Hexabromodiphenyl ether        | 31                                     |              |              |              | 29           | 32    | 30   | 30  | 30 |
| BDE-154                         | 2,2',4,4',5,6'-Hexabromodiphenyl ether        | 30                                     | 31           | 30           | 31           | 30           |       |      |     |    |
| BDE-183                         | 2,2',3,4,4',5',6-Heptabromodiphenyl ether     | 31                                     | 32           | 31           | 29           | 31           |       |      |     |    |
| BDE-209                         | Decabromodiphenyl ether                       | 32                                     |              |              |              | 32           | 32    | 30   | 32  | 31 |
| 2,4,6-TBP*                      | 2,4,6-Tribromophenol                          | 30                                     |              |              | 29           | 32           | 30    | 30   | 30  |    |
| 3-OH-BDE-47*                    | 3-Hydroxy-2,2',4,4'-tetraBDE                  | 30                                     |              | 32           | 29           | 32           | 30    | 30   |     |    |
| 5-OH-BDE-47*                    | 5-Hydroxy-2,2',4,4'-tetraBDE                  | 31                                     |              | 29           | 27           | 31           | 30    | 31   |     |    |
| 6-OH-BDE-47*                    | 6-Hydroxy-2,2',4,4'-tetraBDE                  | 31                                     | 28           | 28           | 29           | 28           | 30    |      |     |    |
| Other Brominated FRs            |                                               |                                        |              |              |              |              |       |      |     |    |
| HBDCD                           | Hexabromocyclododecane (Commercial Mix)       | 30                                     |              |              | 31           | 30           | 30    | 31   | 29  |    |
| TBB                             | 2-ethylhexyl-tetrabromobenzoate               | 31                                     |              |              | 30           | 32           | 32    | 31   | 32  |    |
| TBBPA                           | 3,3',5,5'-Tetrabromobisphenol A               | 31                                     |              |              |              | 32           | 32    | 31   | 24  | #  |
| TBBPA-DBPE                      | TBBPA-2,3-dibromopropyl ether                 | 31                                     |              |              | 30           | 31           | 30    | 32   | 31  |    |
| TBPH                            | Bis(2-ethylhexyl)-tetrabromophthalate         | 32                                     |              | 32           | 29           | 30           | 32    | 31   |     |    |

\*Sample sizes in the 24 hpf PMR assay reflect those embryos that survived with no malformations at 24 hpf.

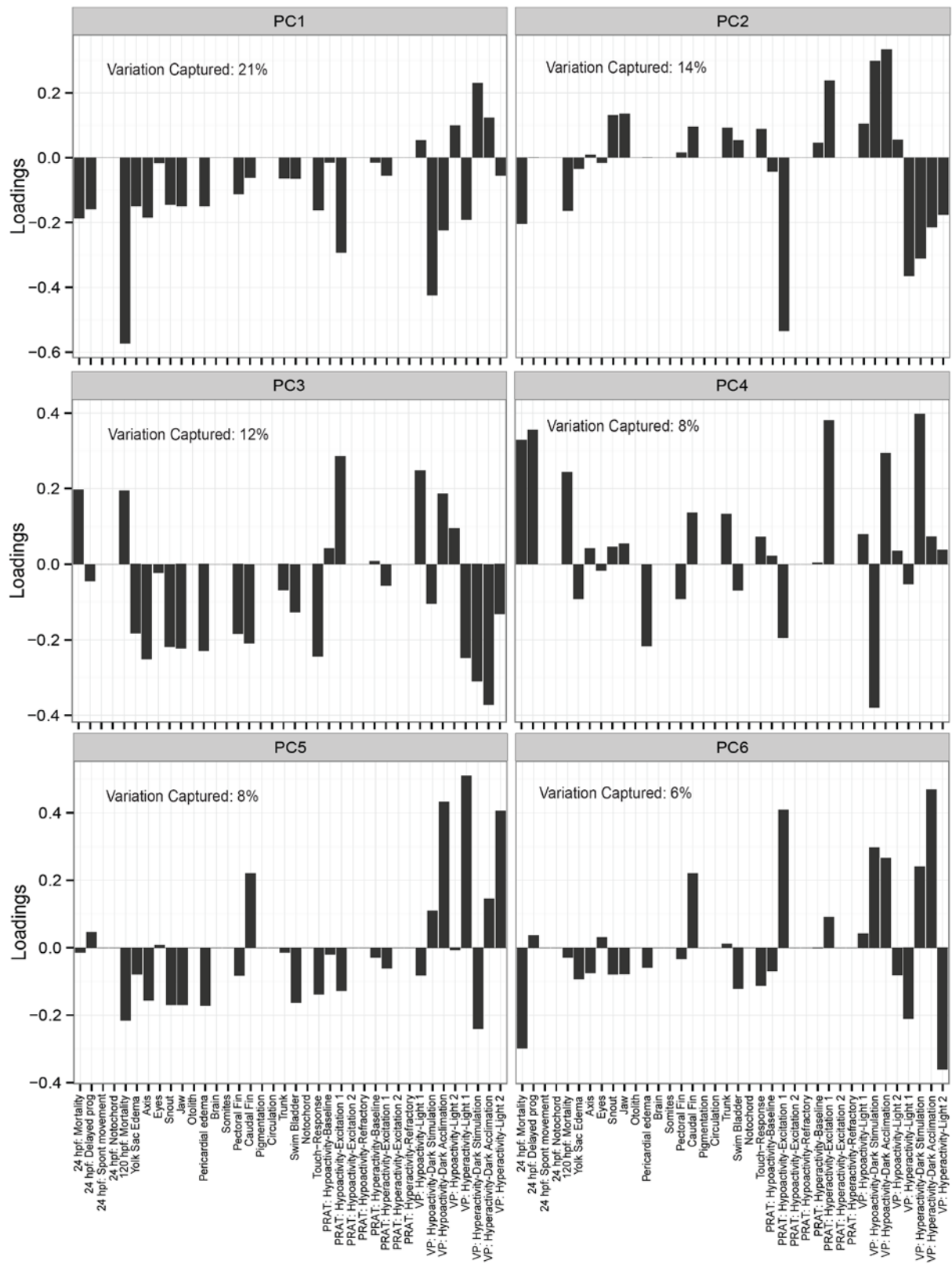
# indicates 100% mortality and/or morbidity.

## 2. Sample Size Larval Photomotor Response Assay at 120 hpf\*

| Abbr                            | Chemical                                      | Sample size by test concentration (µM) |              |              |              |              |       |      |     |    |
|---------------------------------|-----------------------------------------------|----------------------------------------|--------------|--------------|--------------|--------------|-------|------|-----|----|
|                                 |                                               | 0                                      | 6.40<br>E-06 | 6.40<br>E-05 | 6.40<br>E-04 | 6.40<br>E-03 | 0.064 | 0.64 | 6.4 | 64 |
| Chlorinated Phosphate Ester FRs |                                               |                                        |              |              |              |              |       |      |     |    |
| BDP                             | bisphenol A bis-(diphenyl phosphate)          | 31                                     |              |              | 26           | 26           | 24    | 27   | 25  |    |
| BPDP                            | <i>t</i> -Butylphenyl diphenyl phosphate      | 31                                     |              |              |              | 28           | 26    | 28   | 29  | #  |
| EHDP                            | 2-Ethylhexyl diphenyl phosphate               | 28                                     |              |              |              | 26           | 28    | 32   | 30  | 19 |
| IDDP                            | Isodecyl diphenyl phosphate                   | 30                                     |              |              |              | 27           | 27    | 29   | 28  | 23 |
| IPP-1                           | Isopropylated triphenyl phosphate - 1         | 29                                     |              |              |              | 31           | 28    | 32   | 28  | #  |
| IPP-2                           | Isopropylated triphenyl phosphate - 2         | 28                                     |              |              |              | 26           | 27    | 25   | 24  | 6  |
| IPP-3                           | Isopropylated triphenyl phosphate - 3         | 29                                     |              |              |              | 30           | 27    | 26   | 25  | #  |
| mITP                            | Mono isopropylated triaryl phosphate          | 31                                     |              | 30           | 30           | 28           | 27    | 3    |     |    |
| RDP                             | Resorcinol A bis-(diphenyl phosphate)         | 30                                     |              |              | 29           | 30           | 32    | 27   | 27  |    |
| TBP                             | Tributyl phosphate                            | 28                                     |              |              | 24           | 23           | 22    | 29   | 28  |    |
| TBEP                            | Tris (2-butoxyethyl) phosphate                | 30                                     |              |              | 30           | 29           | 28    | 28   | 28  |    |
| TCP                             | Tricresyl phosphate                           | 28                                     |              |              |              | 23           | 24    | 24   | 24  | 7  |
| o-TCP                           | Tri- <i>o</i> -cresyl phosphate               | 28                                     |              |              |              | 24           | 28    | 27   | 28  | 11 |
| TEHP                            | Tris (2-ethylhexyl) phosphate                 | 29                                     |              |              | 28           | 28           | 30    | 24   | 24  |    |
| TPP                             | Triphenyl phosphate                           | 27                                     |              |              |              | 23           | 23    | 24   | 27  | 19 |
| DPP*                            | Diphenyl phosphate                            | 30                                     |              |              | 30           | 29           | 30    | 30   | 32  |    |
| Chlorinated Phosphate Ester FRs |                                               |                                        |              |              |              |              |       |      |     |    |
| BCPCP*                          | Bis (2-chloropropyl) 1-carboxyethyl phosphate | 30                                     |              |              |              | 32           | 28    | 29   | 28  | 29 |
| BCPP*                           | Bis(1,3-dichloro-2-propyl)phosphate           | 30                                     |              |              |              | 30           | 27    | 31   | 30  | 31 |
| BDCPP*                          | Bis (2-chloropropyl) phosphate                | 31                                     |              |              |              | 22           | 27    | 23   | 28  | 26 |
| Dechlorane                      | Dechlorane Plus                               | 30                                     |              |              | 29           | 26           | 28    | 28   | 30  |    |
| MCPP*                           | Mono (2-chloropropyl) phosphate               | 31                                     |              |              |              | 30           | 31    | 29   | 28  | 31 |
| TCEP                            | Tris (1-chloropropyl) phosphate               | 31                                     |              |              |              | 26           | 30    | 26   | 31  | 30 |
| TCPP                            | Tris (2-chloroethyl) phosphate                | 29                                     |              |              |              | 32           | 28    | 30   | 30  | 27 |
| TDCPP                           | Tris (1,3-dichloro-2-propyl) phosphate        | 28                                     |              |              |              | 31           | 29    | 30   | 27  | 12 |
| PBDE FRs                        |                                               |                                        |              |              |              |              |       |      |     |    |
| DE-71                           | PentaBDE commercial mixture                   | 27                                     |              |              |              | 31           | 23    | 28   | 28  | 16 |
| DE-79                           | OctaBDE commercial mixture                    | 30                                     |              |              |              | 29           | 29    | 29   | 31  | 29 |
| BDE-3                           | <i>p</i> -Bromodiphenyl ether                 | 30                                     |              |              |              | 29           | 29    | 29   | 30  | 29 |
| BDE-15                          | <i>p,p</i> -Dibromodiphenyl ether             | 28                                     |              |              |              | 30           | 28    | 24   | 28  | 22 |
| BDE-47                          | 2,2',4,4'-Tetrabromodiphenyl ether            | 29                                     |              |              |              | 22           | 24    | 23   | 26  | 18 |
| BDE-99                          | 2,2',4,4',5-Pentabromodiphenyl ether          | 30                                     |              |              |              | 29           | 25    | 25   | 25  | 28 |
| BDE-100                         | 2,2',4,4',6-Pentabromodiphenyl ether          | 29                                     | 28           | 25           | 30           | 27           |       |      |     |    |
| BDE-153                         | 2,2',4,4',5,5'-Hexabromodiphenyl ether        | 31                                     |              |              |              | 25           | 31    | 27   | 30  | 30 |
| BDE-154                         | 2,2',4,4',5,6'-Hexabromodiphenyl ether        | 31                                     | 30           | 29           | 28           | 28           |       |      |     |    |
| BDE-183                         | 2,2',3,4,4',5',6-Heptabromodiphenyl ether     | 31                                     | 28           | 29           | 28           | 30           |       |      |     |    |
| BDE-209                         | Decabromodiphenyl ether                       | 32                                     |              |              |              | 32           | 32    | 30   | 32  | 31 |
| 2,4,6-TBP*                      | 2,4,6-Tribromophenol                          | 30                                     |              |              | 26           | 26           | 24    | 27   | 25  |    |
| 3-OH-BDE-47*                    | 3-Hydroxy-2,2',4,4'-tetraBDE                  | 29                                     |              | 31           | 29           | 31           | 29    | 29   |     |    |
| 5-OH-BDE-47*                    | 5-Hydroxy-2,2',4,4'-tetraBDE                  | 27                                     |              | 27           | 23           | 26           | 27    | 28   |     |    |
| 6-OH-BDE-47*                    | 6-Hydroxy-2,2',4,4'-tetraBDE                  | 28                                     | 24           | 26           | 27           | 25           | 25    |      |     |    |
| Other Brominated FRs            |                                               |                                        |              |              |              |              |       |      |     |    |
| HBDCD                           | Hexabromocyclododecane (Commercial Mix)       | 29                                     |              |              | 31           | 29           | 27    | 30   | 23  |    |
| TBB                             | 2-ethylhexyl-tetrabromobenzoate               | 31                                     |              |              | 29           | 30           | 31    | 30   | 32  |    |
| TBBPA                           | 3,3',5,5'-Tetrabromobisphenol A               | 31                                     |              |              |              | 29           | 29    | 27   | #   | #  |
| TBBPA-DBPE                      | TBBPA-2,3-dibromopropyl ether                 | 29                                     |              |              | 30           | 28           | 27    | 30   | 29  |    |
| TBPH                            | Bis(2-ethylhexyl)-tetrabromophthalate         | 32                                     |              | 31           | 28           | 29           | 32    | 31   |     |    |

\*Sample sizes in the 120 hpf PMR assay reflect larvae that survived with no malformations detected at either 24 hpf or 120 hpf. # indicates 100% mortality and/or morbidity.

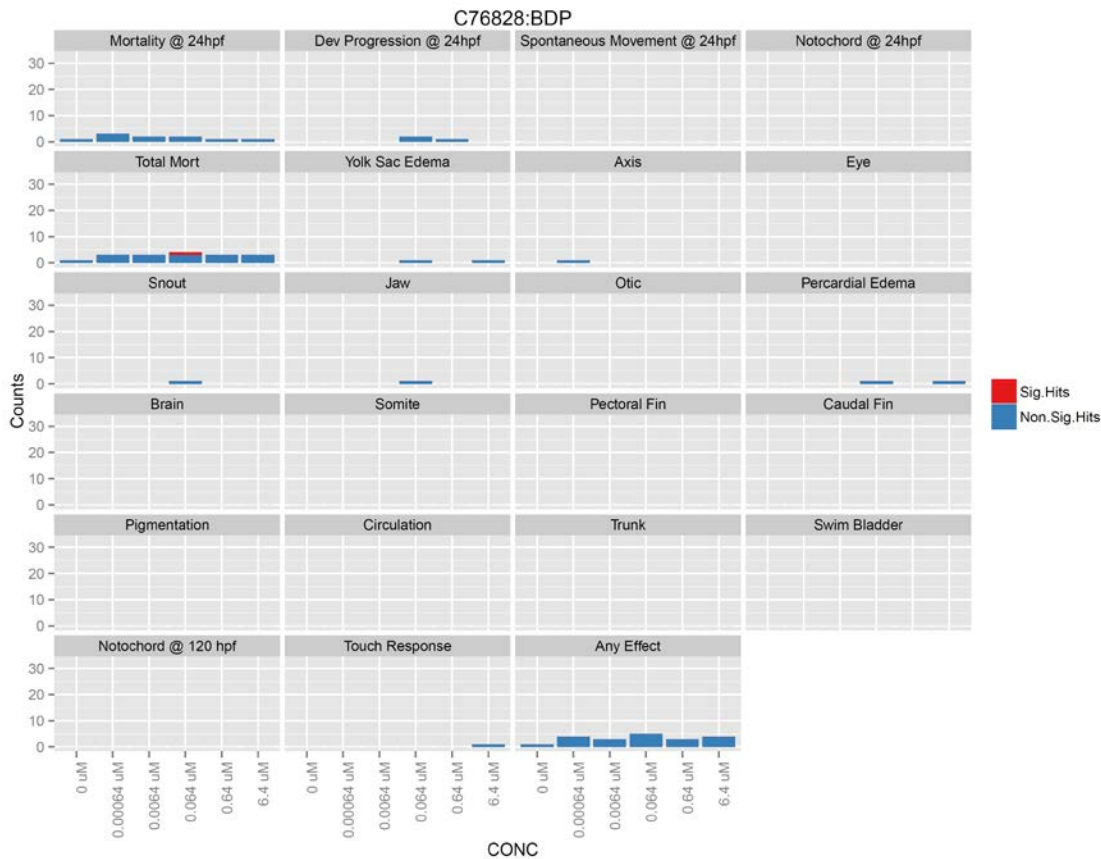
### 3. PCA Loadings



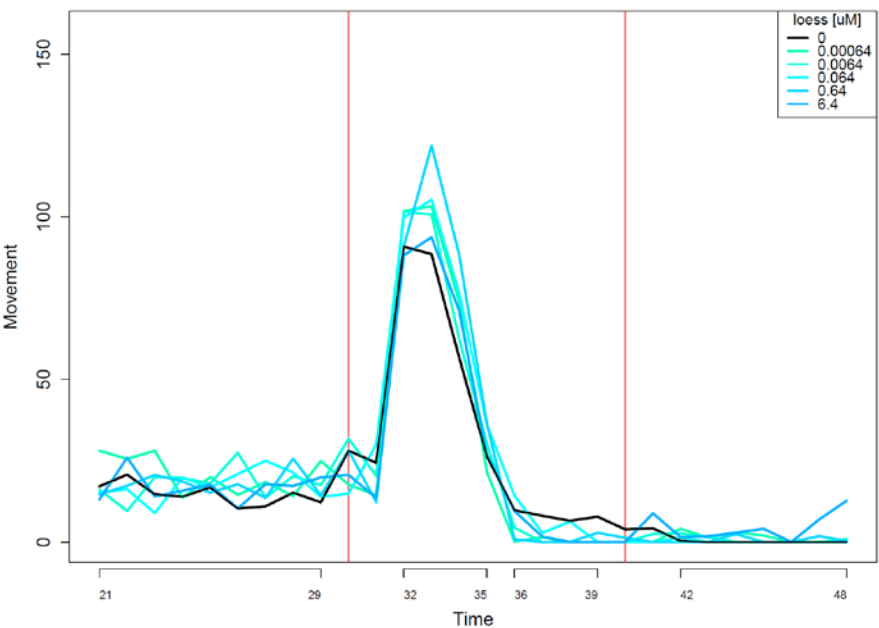
4. ARYL PHOSPHATE ESTER (APE) FLAME RETARDANTS

Bisphenol A bis-(diphenyl phosphate) (BDP)

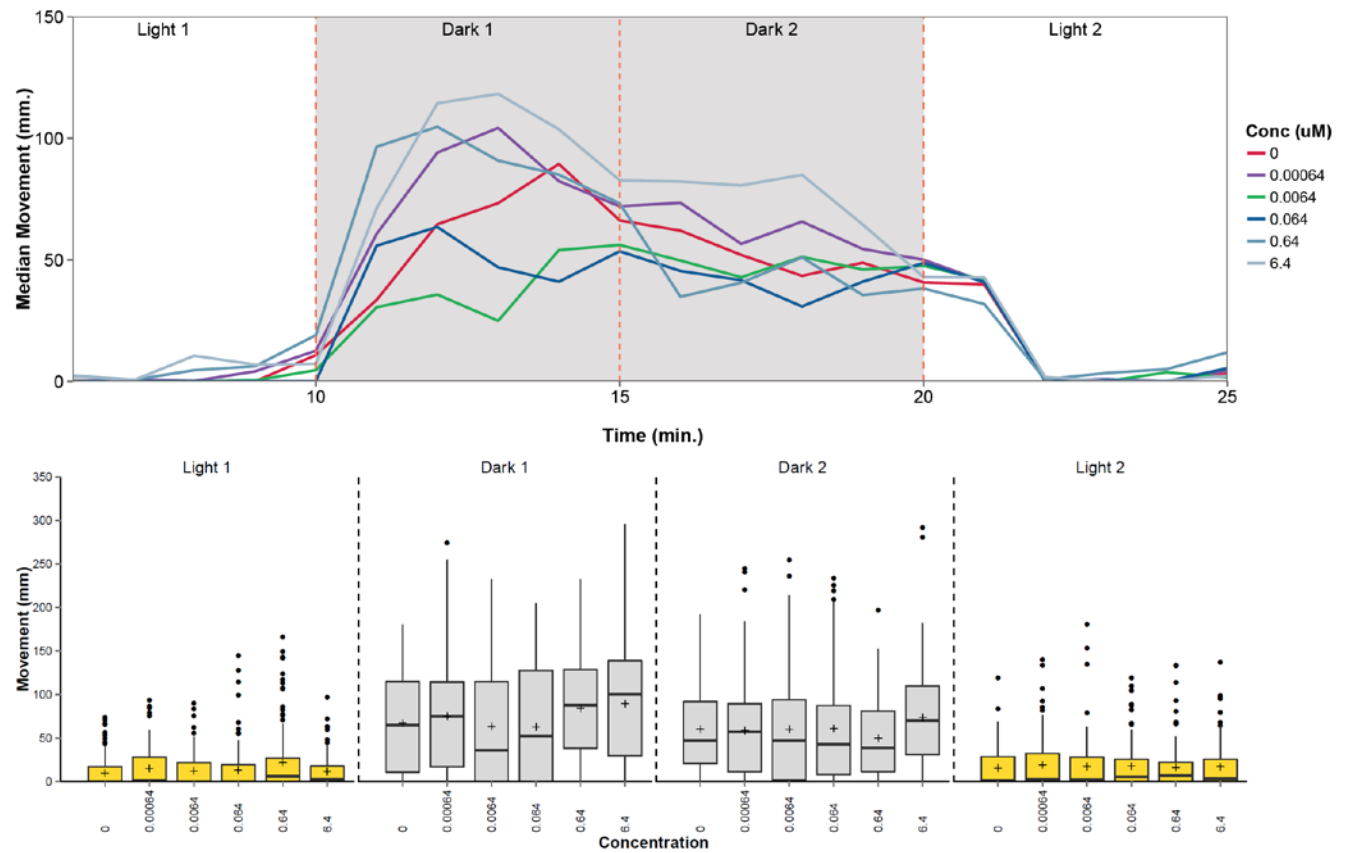
*BDP: Zebrafish Mortality and Malformations at 24 and 120 hpf*



*BDP: Zebrafish embryo PRAT results at 24 hpf*

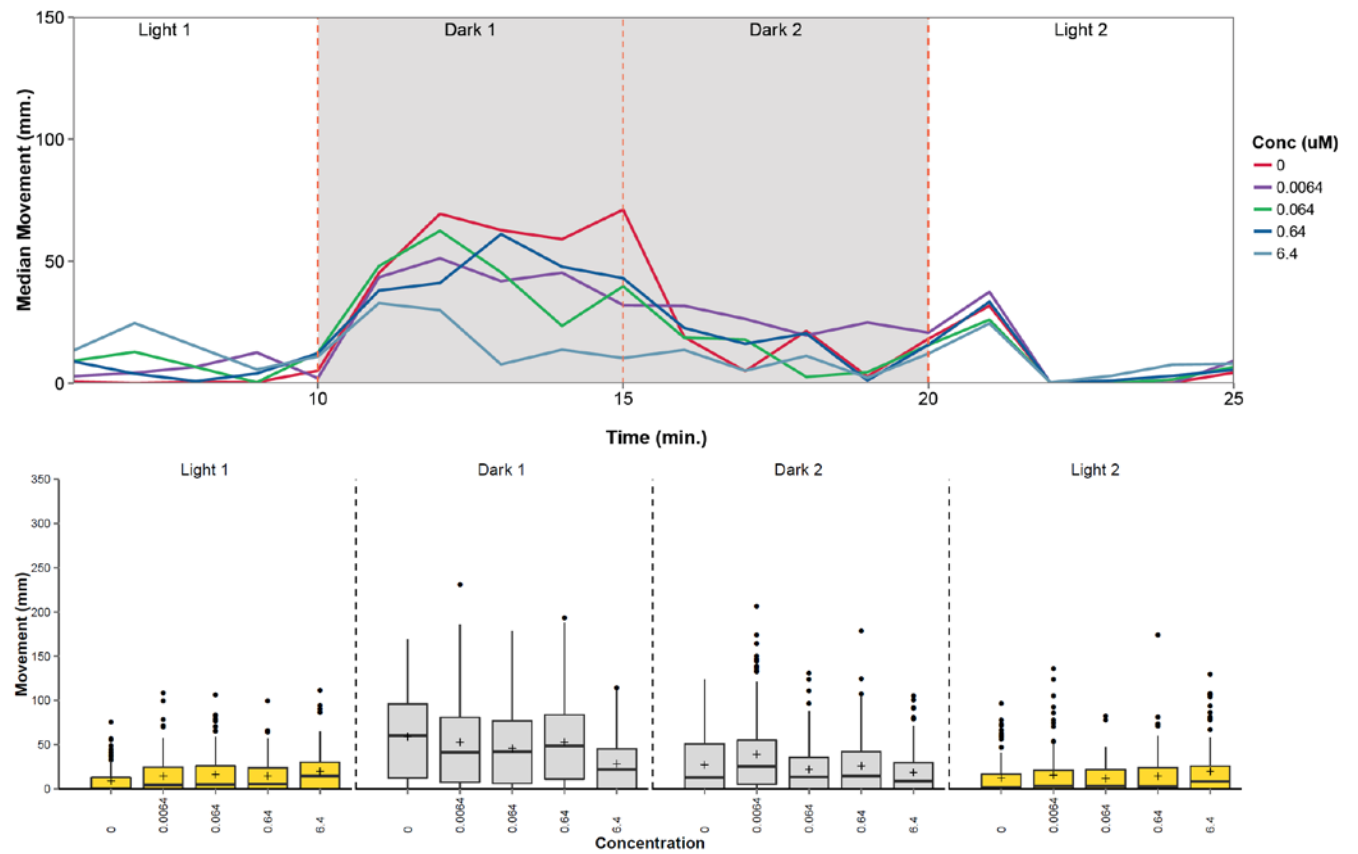


**BDP: Zebrafish larvae PMR assay results at 120 hpf**



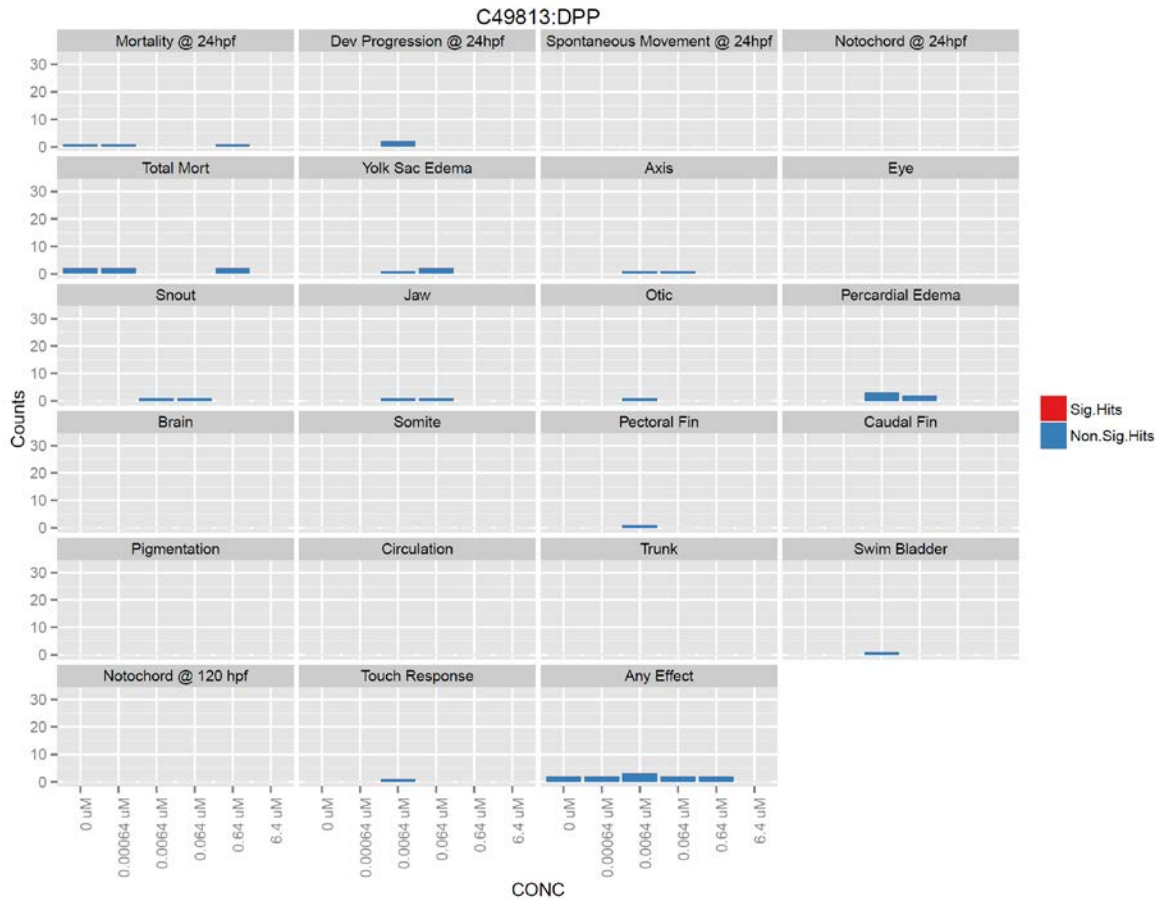


**BPDP: Zebrafish larvae PMR assay results at 120 hpf**

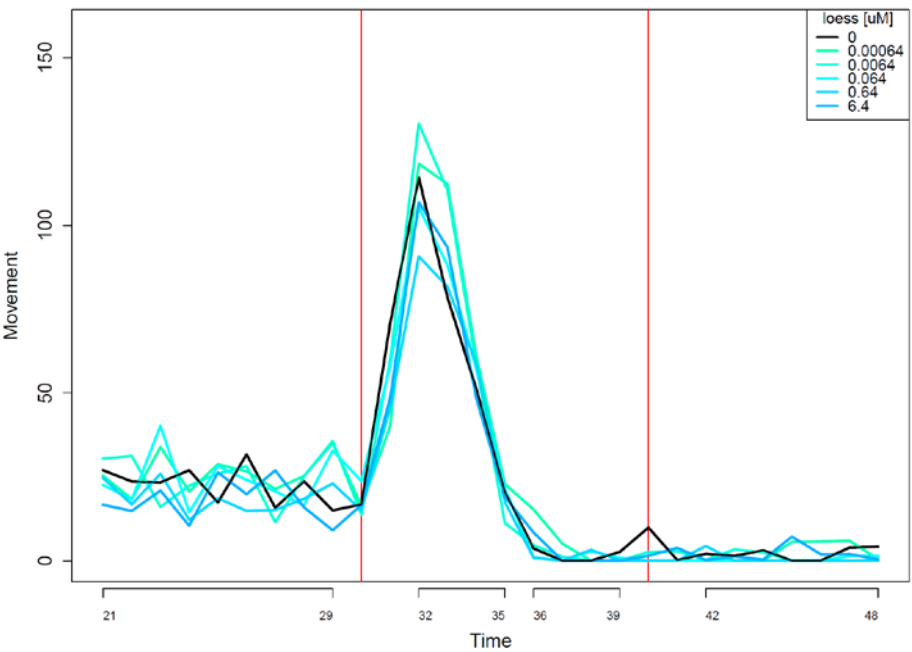


**Diphenyl phosphate (DPP)**

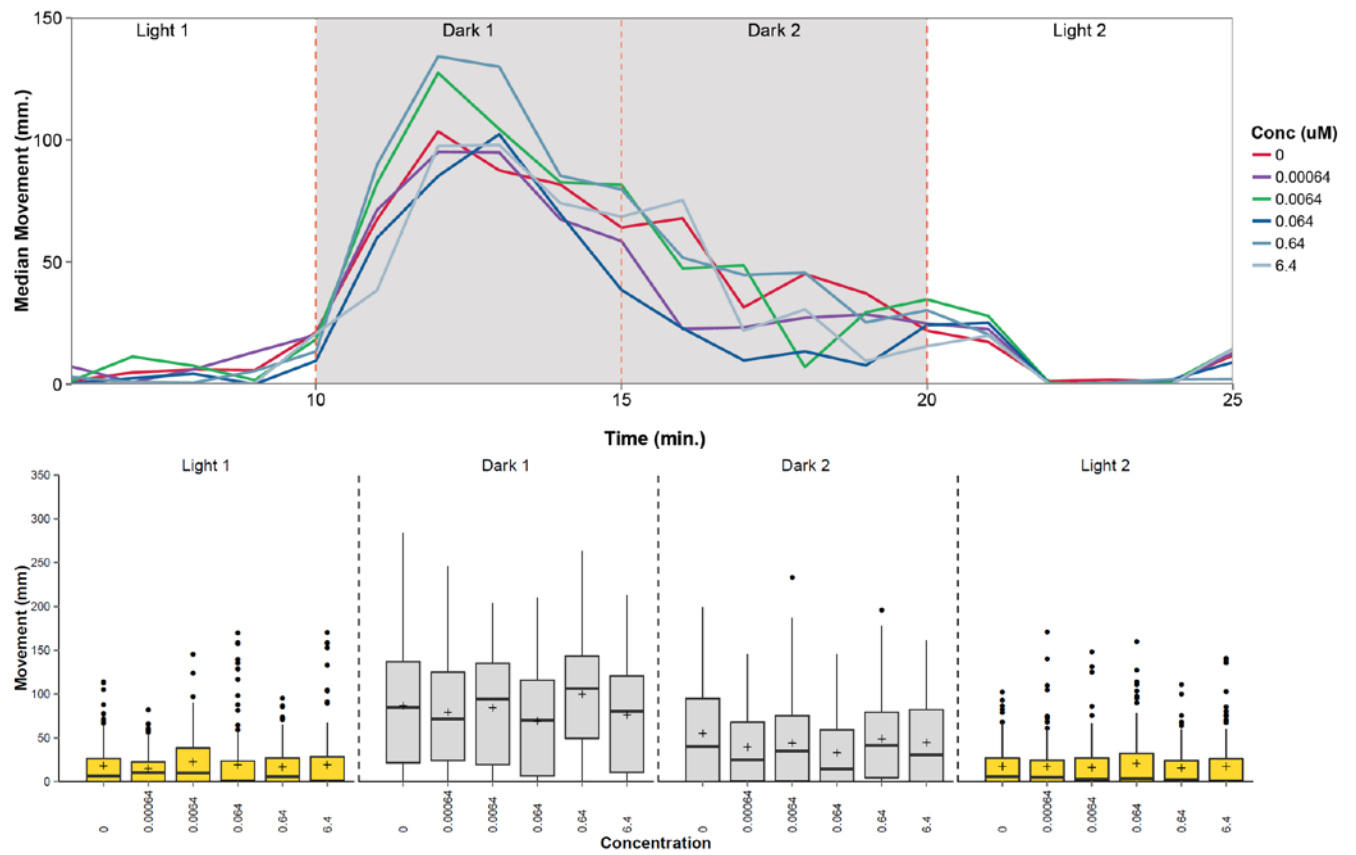
**DPP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**DPP: Zebrafish embryo PRAT results at 24 hpf**

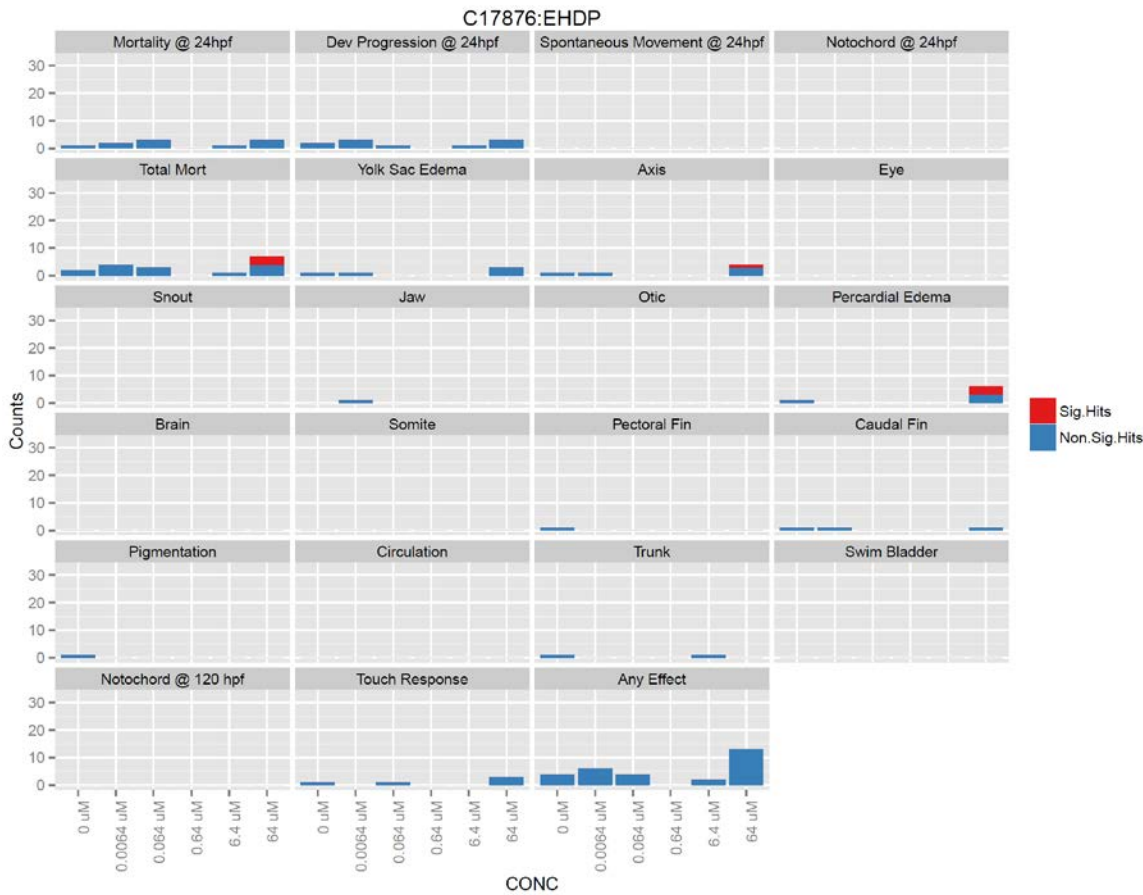


**DPP: Zebrafish larvae PMR assay results at 120 hpf**

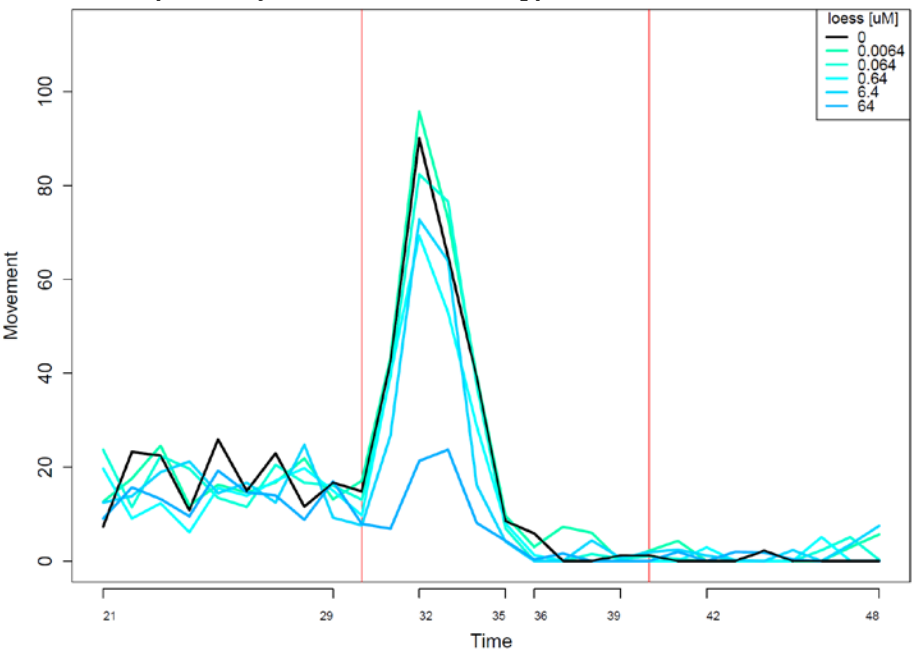


**2-Ethylhexyl diphenyl phosphate (EHDP)**

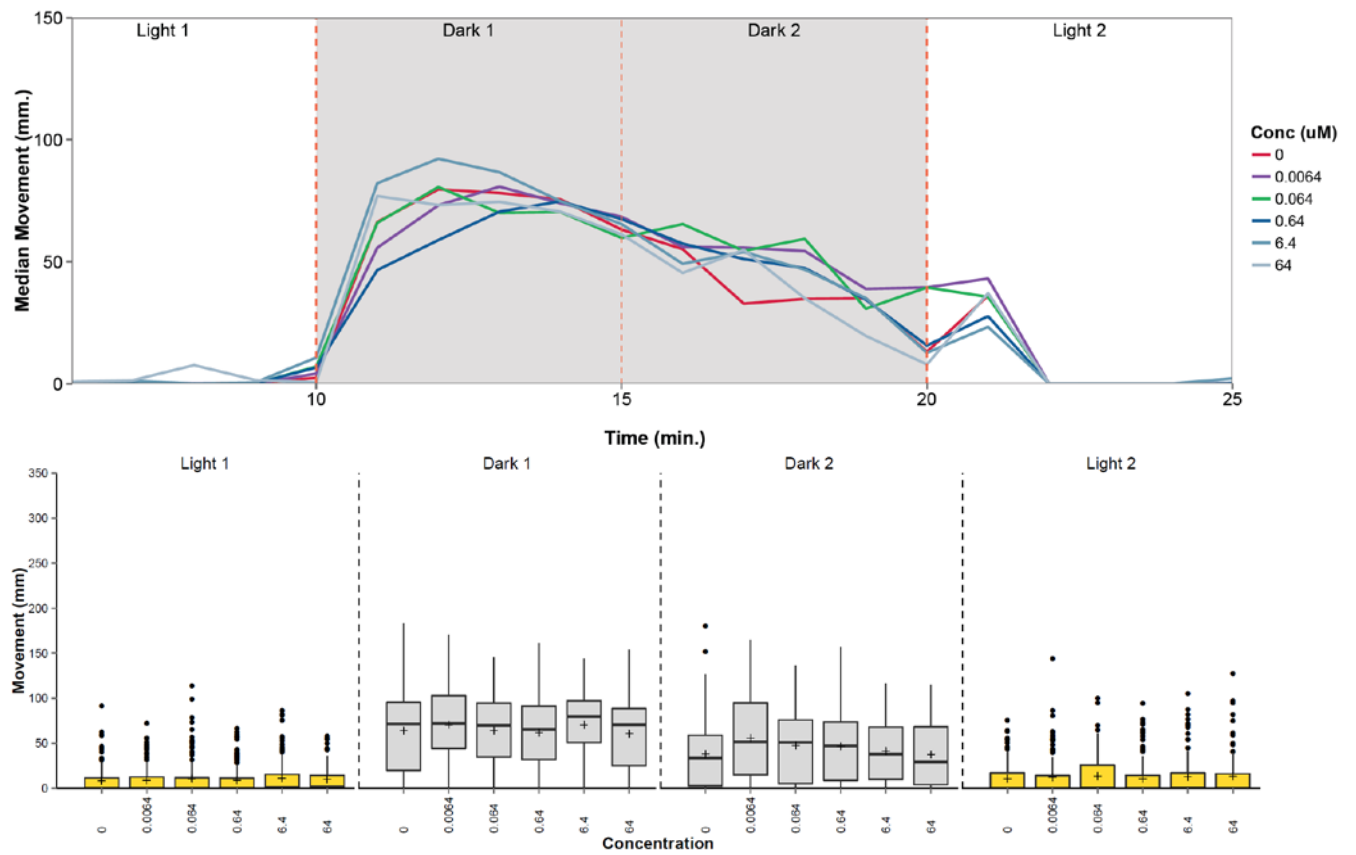
***EHDP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***EHDP: Zebrafish embryo PRAT results at 24 hpf***

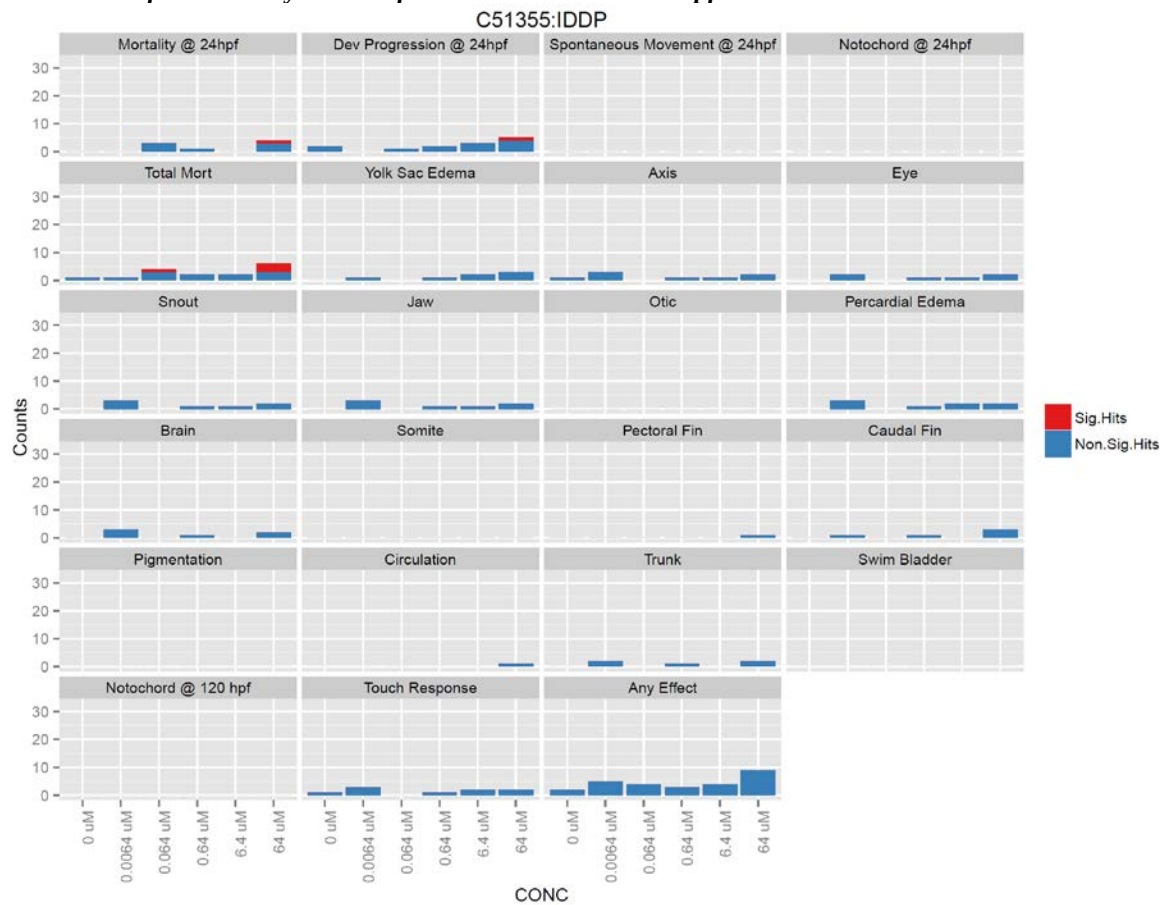


***EHDP: Zebrafish larvae PMR assay results at 120 hpf***

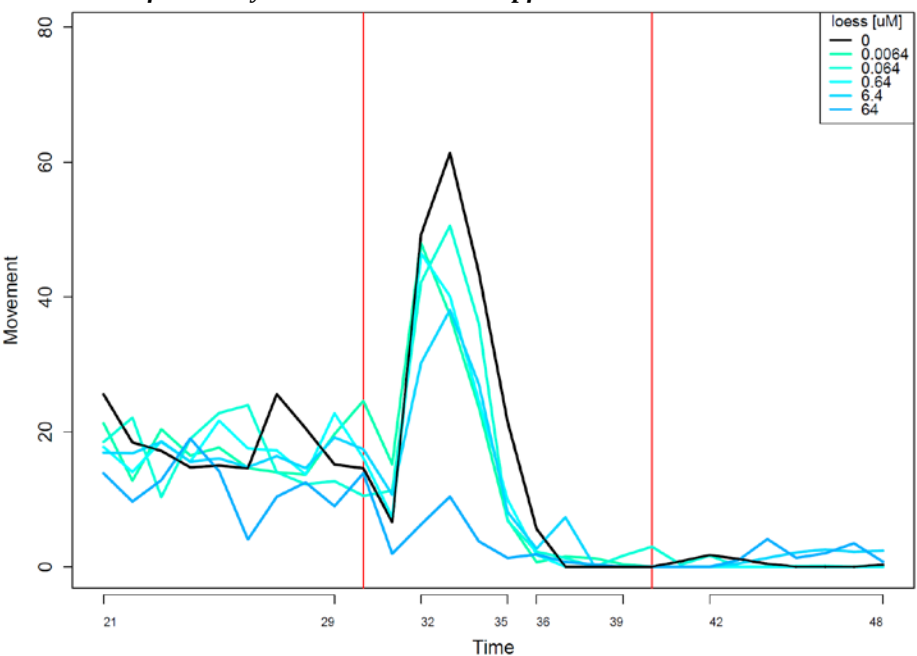


**Isodecyl diphenyl phosphate (IDDP)**

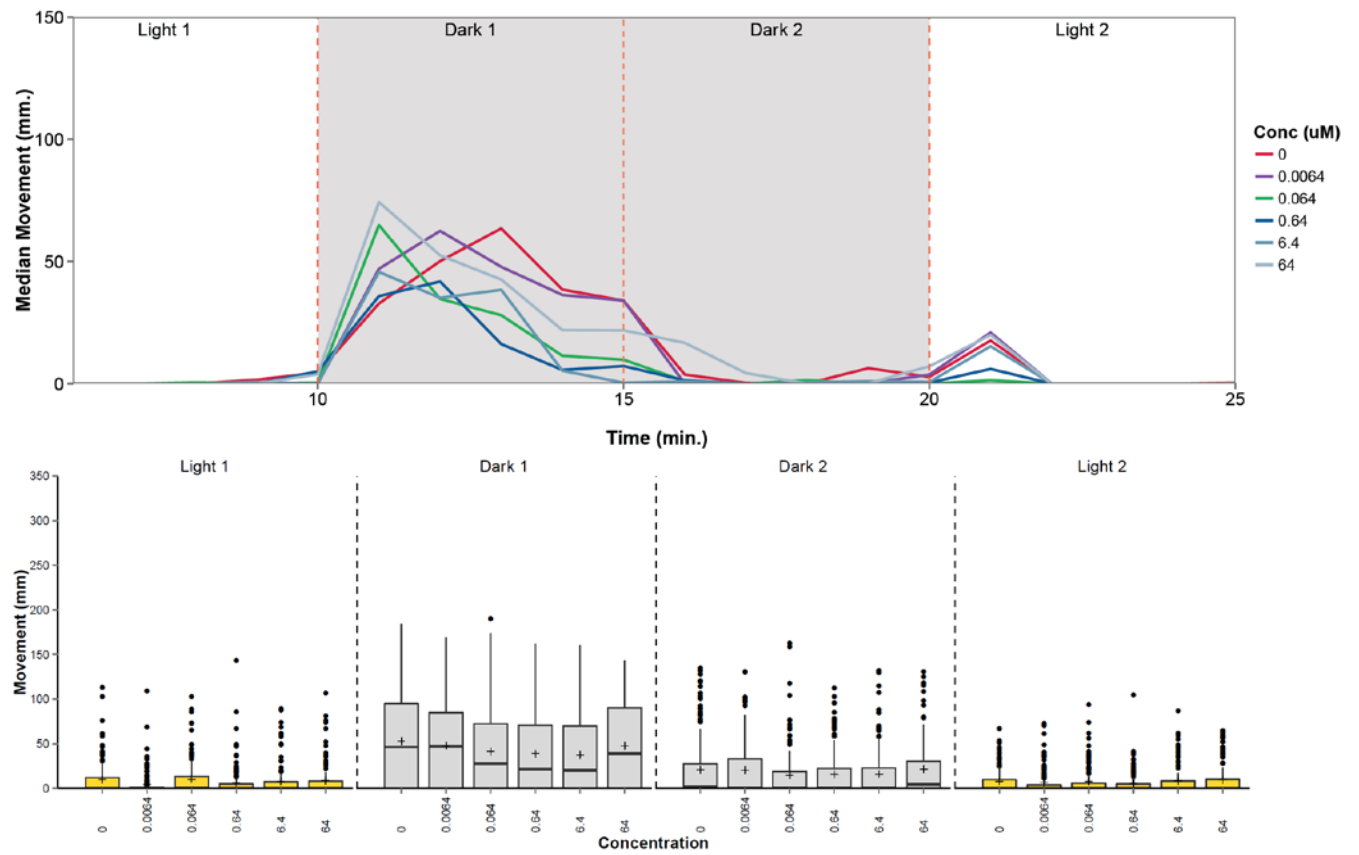
***IDDP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***IDDP: Zebrafish embryo PRAT results at 24 hpf***

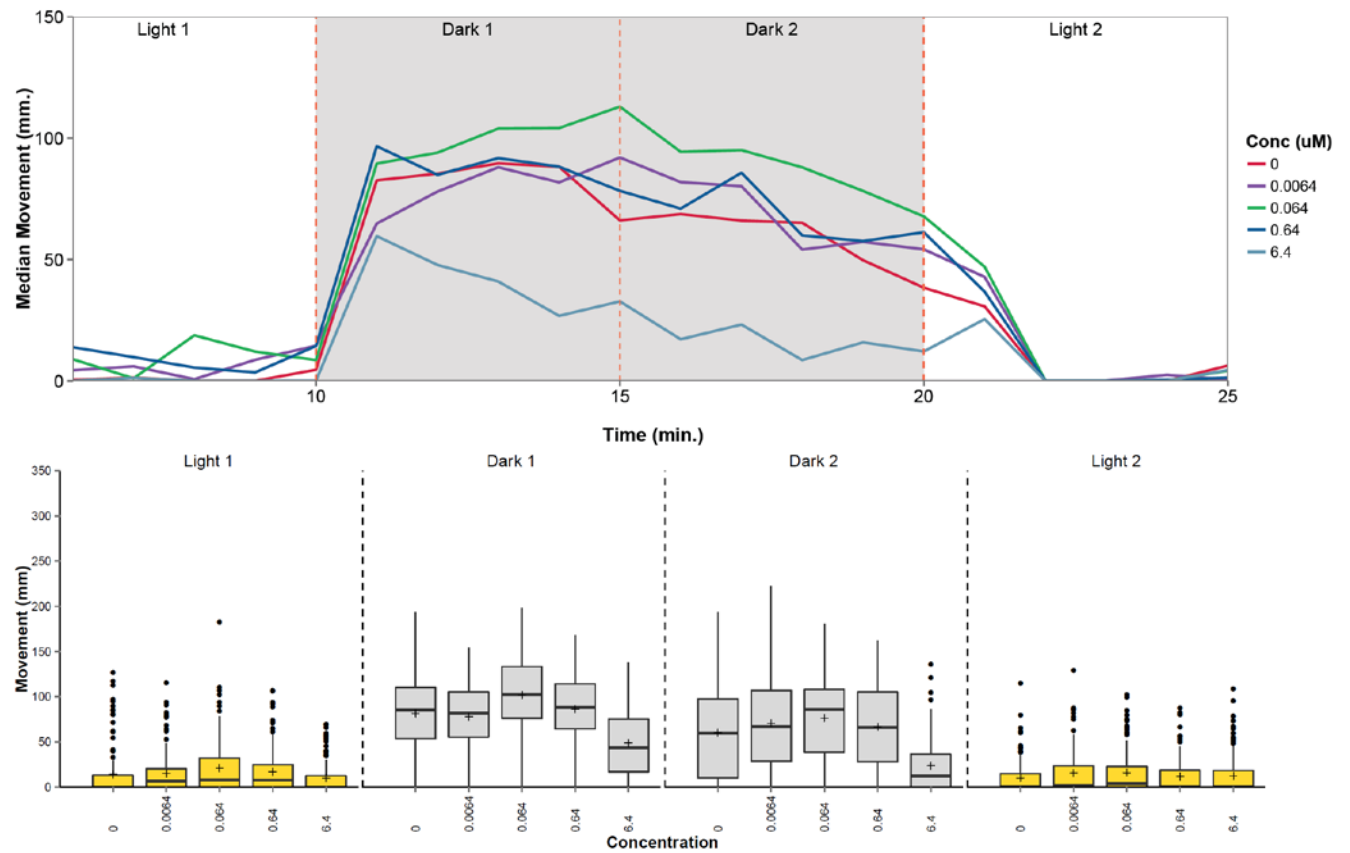


**IDDP: Zebrafish larvae PMR assay results at 120 hpf**



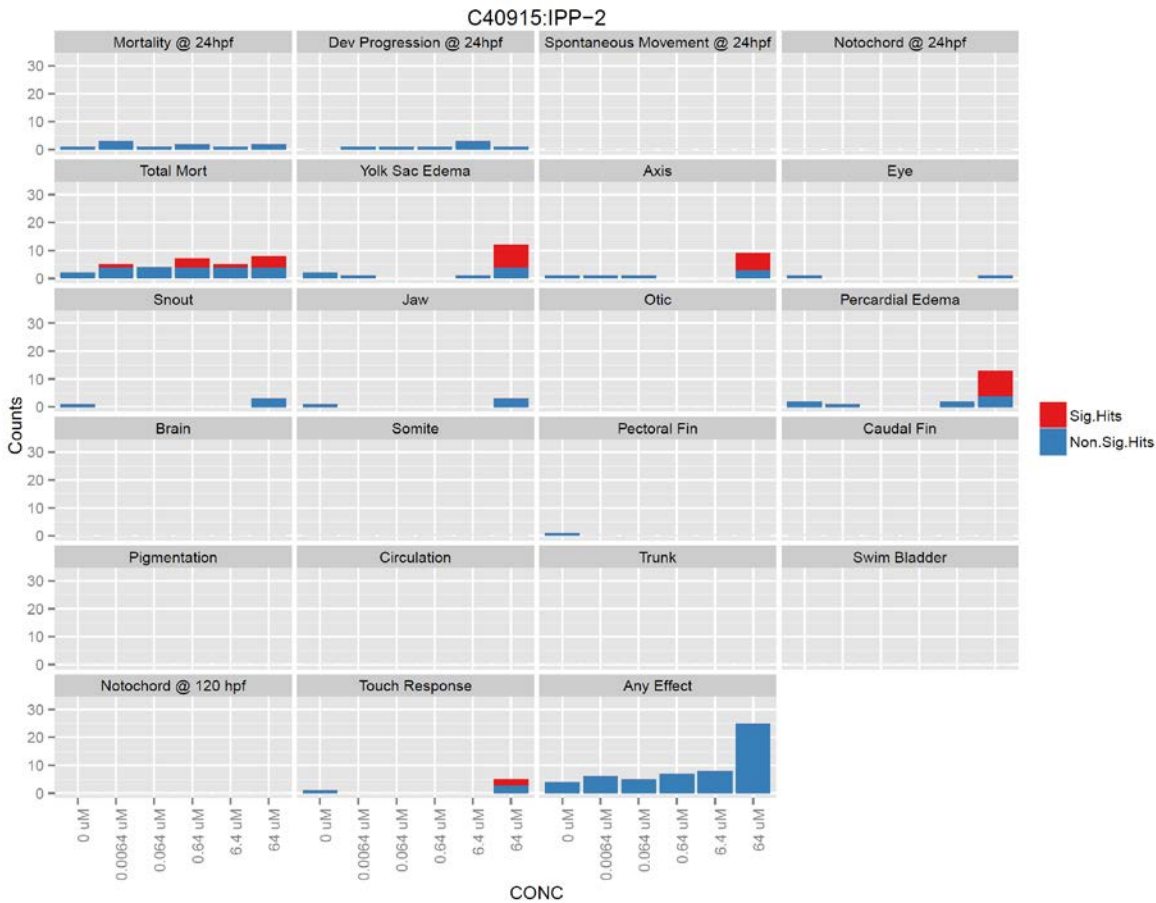


**IPP-1: Zebrafish larvae PMR assay results at 120 hpf**

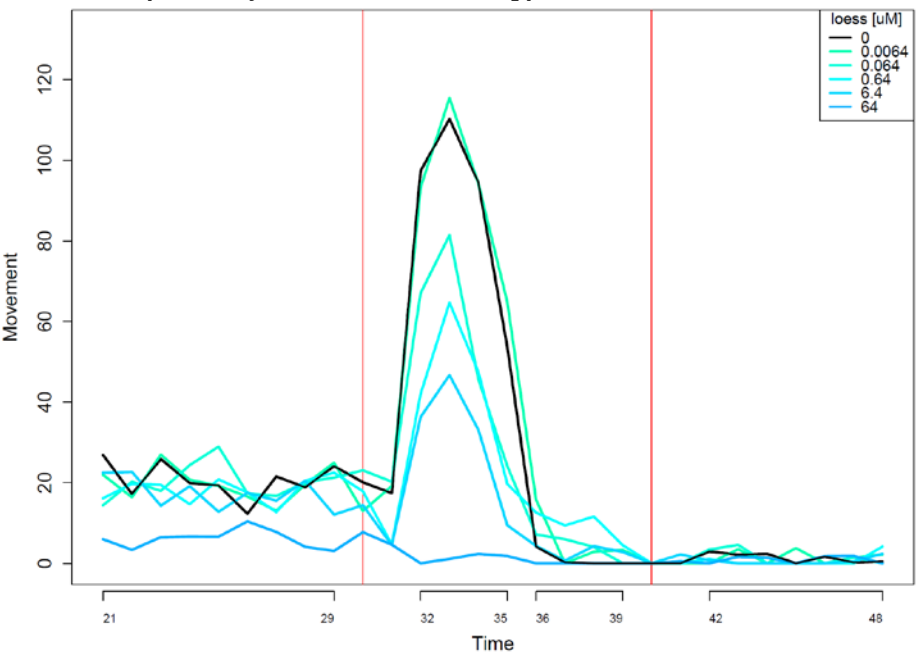


**Isopropylated triphenyl phosphate-2 (IPP-2)**

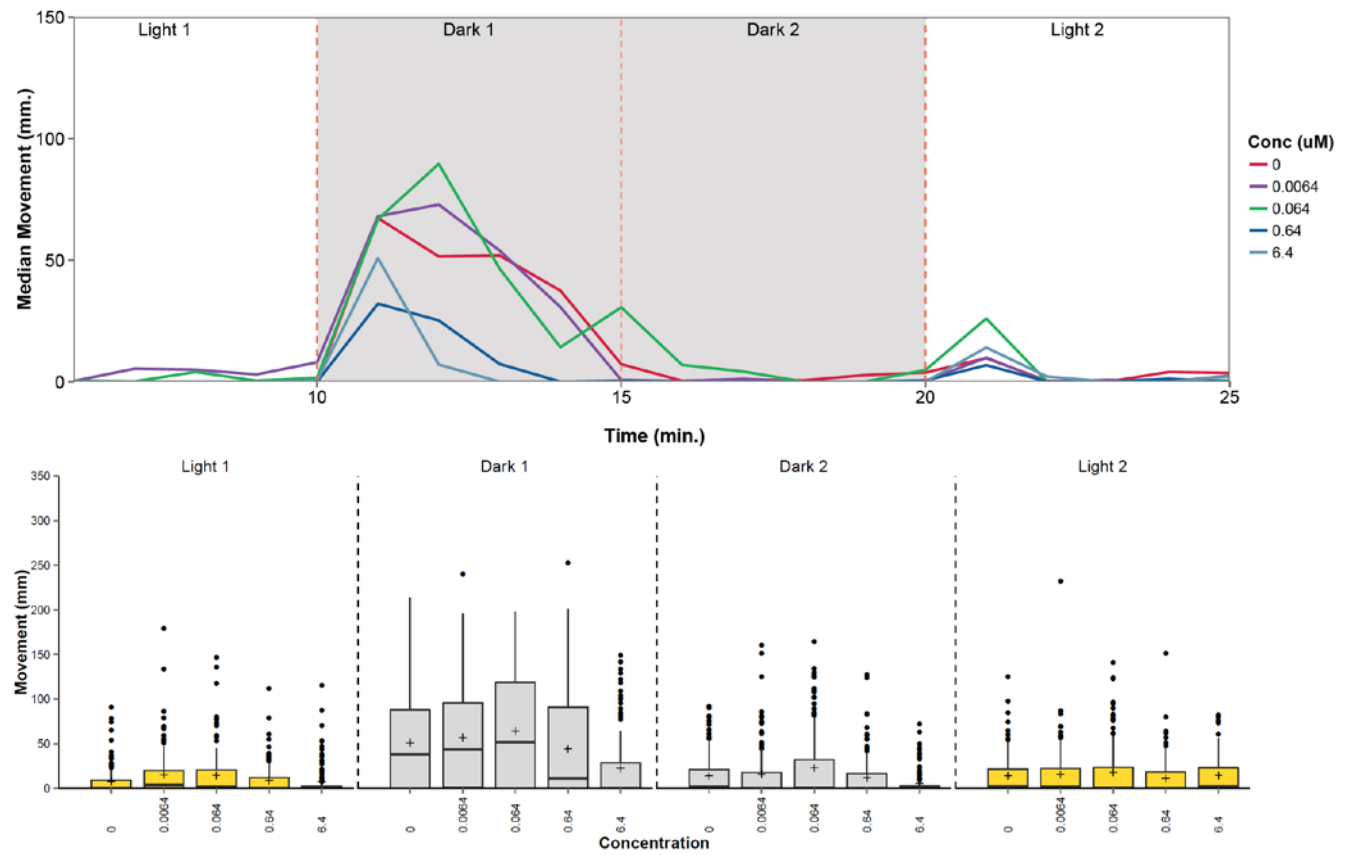
***IPP-2: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***IPP-2: Zebrafish embryo PRAT results at 24 hpf***

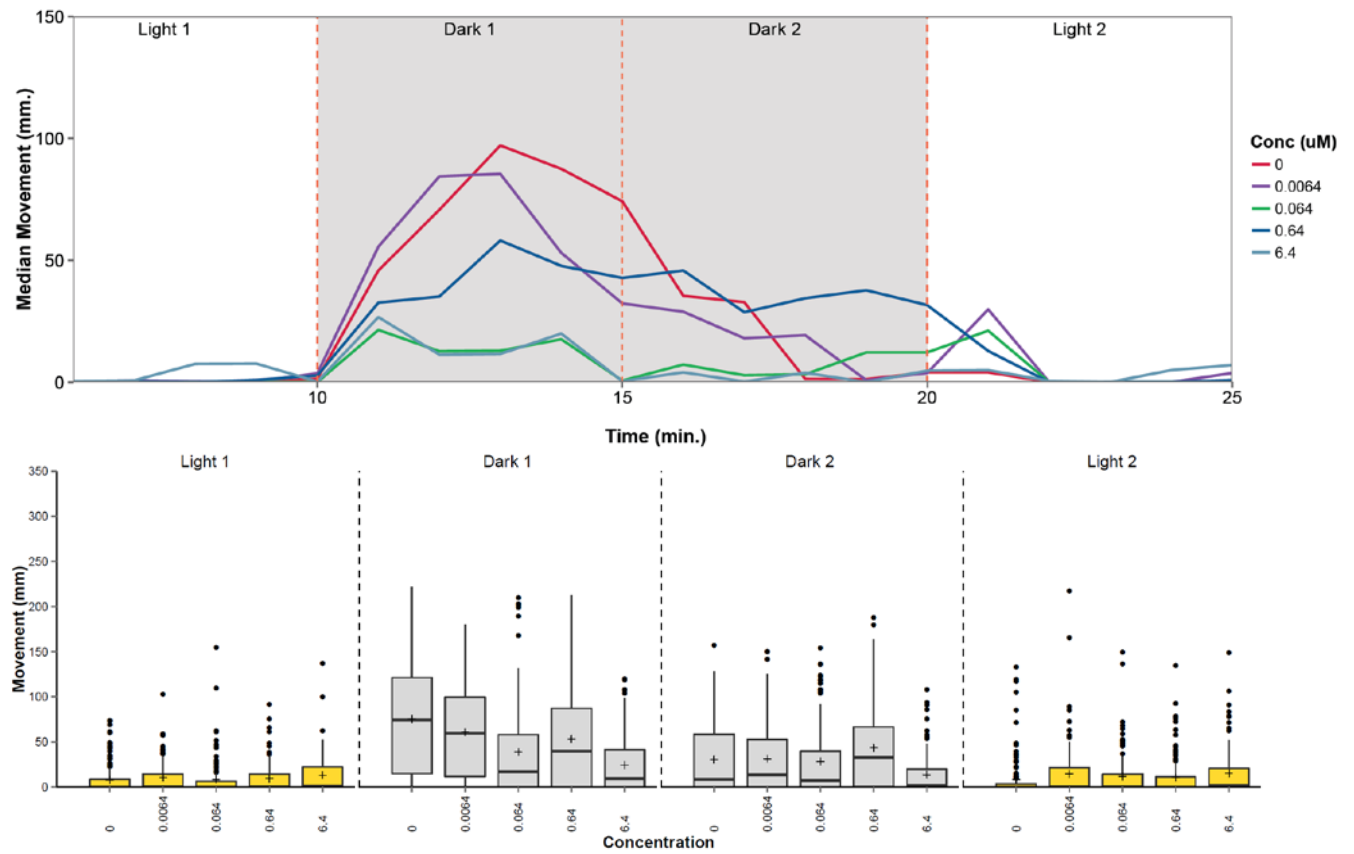


**IPP-2: Zebrafish larvae PMR assay results at 120 hpf**



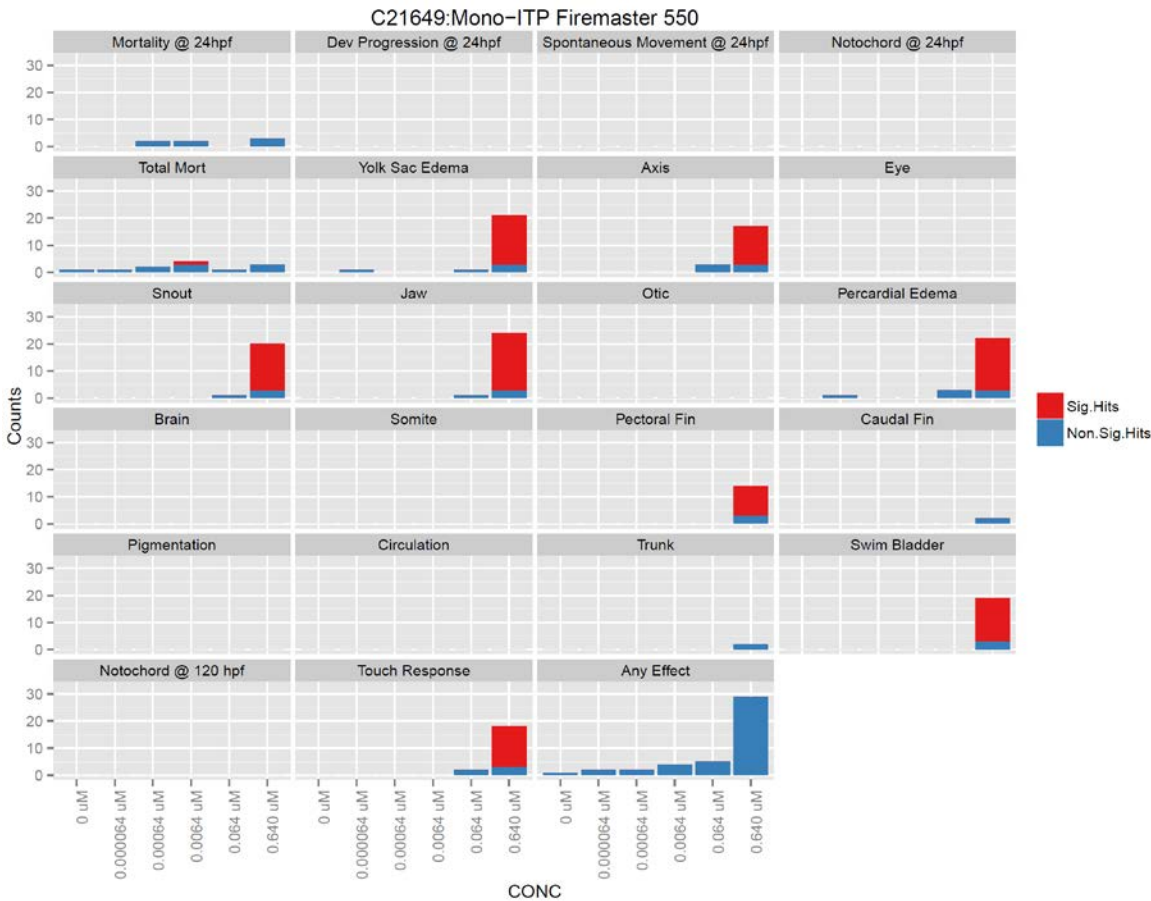


**IPP-3: Zebrafish larvae PMR assay results at 120 hpf**

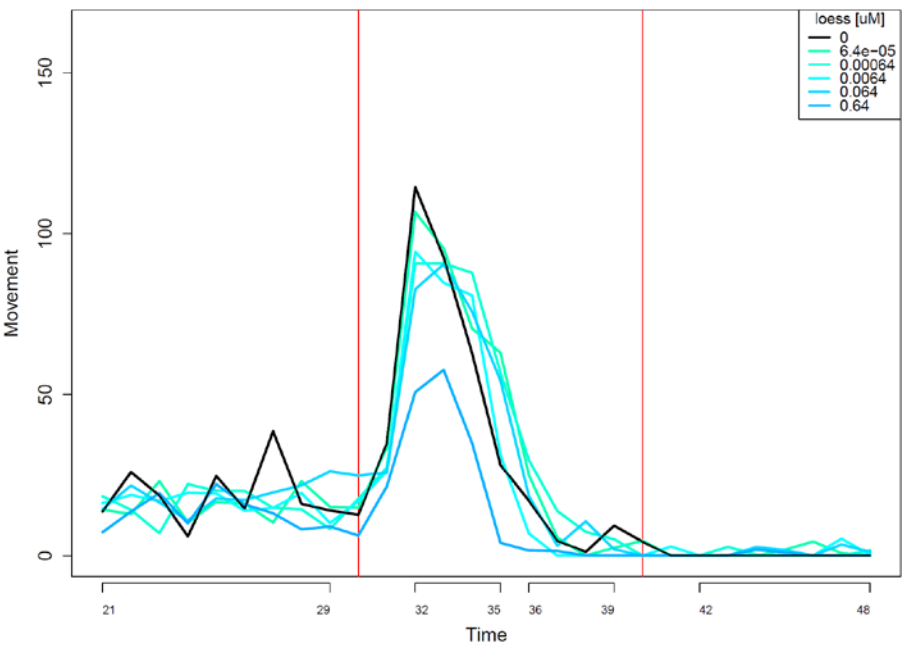


**Mono-substituted isopropylated triaryl phosphate (ortho-, meta, para mix) (mITP)**

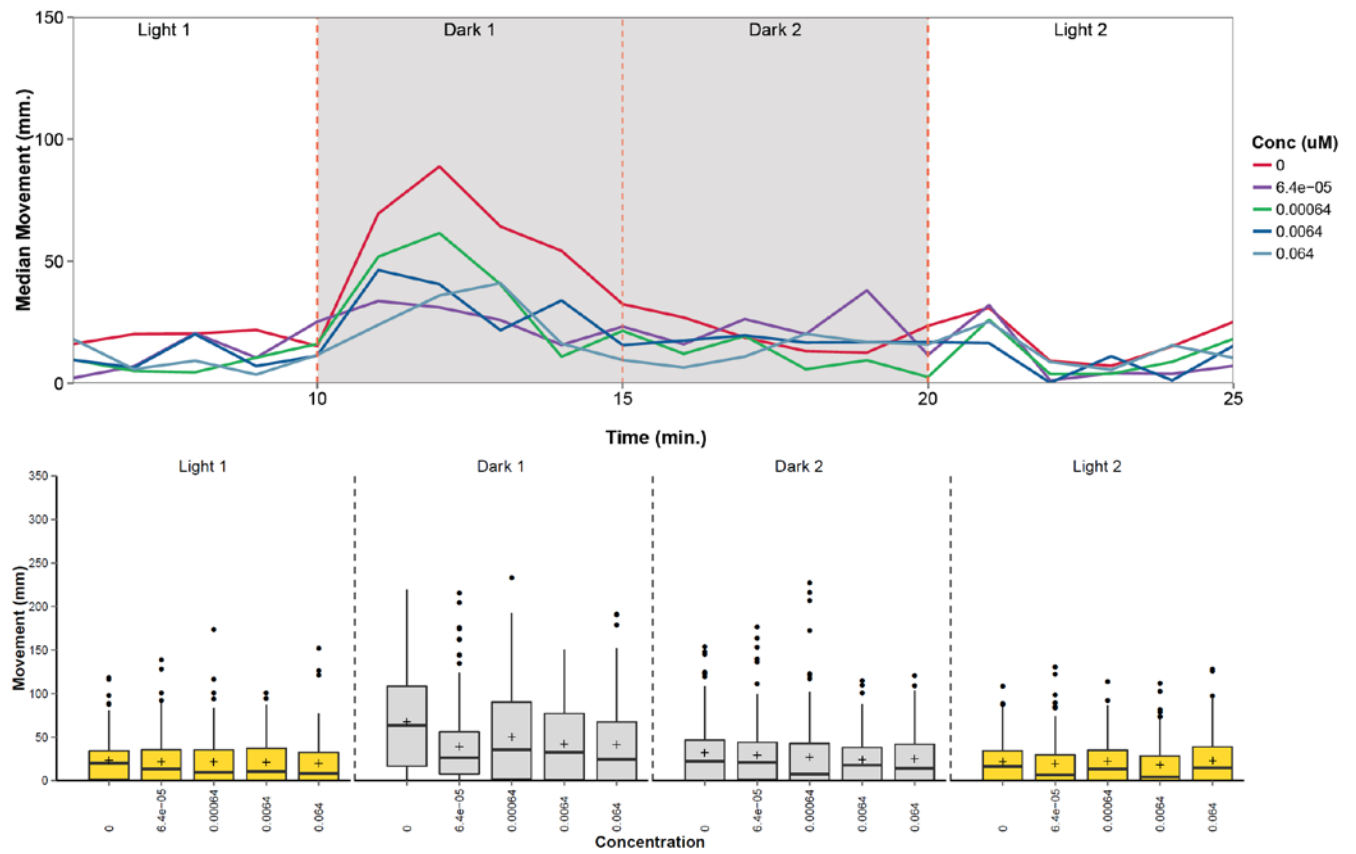
***mITP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



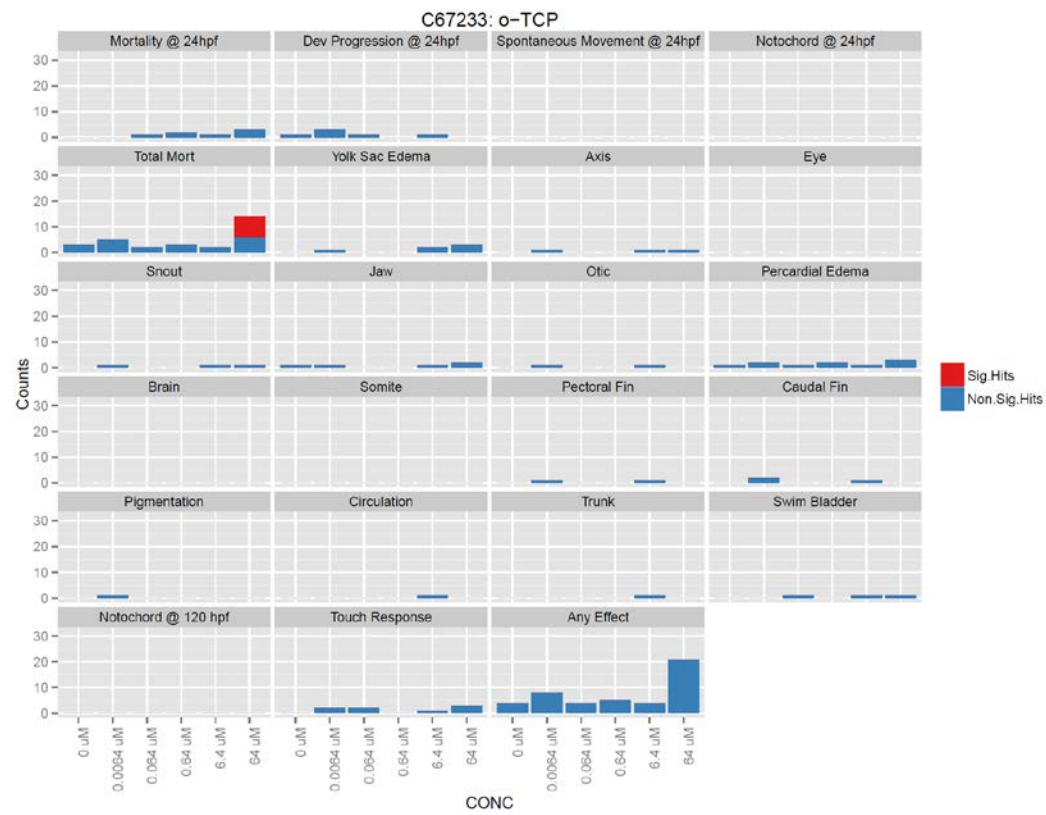
***mITP: Zebrafish embryo PRAT results at 24 hpf***



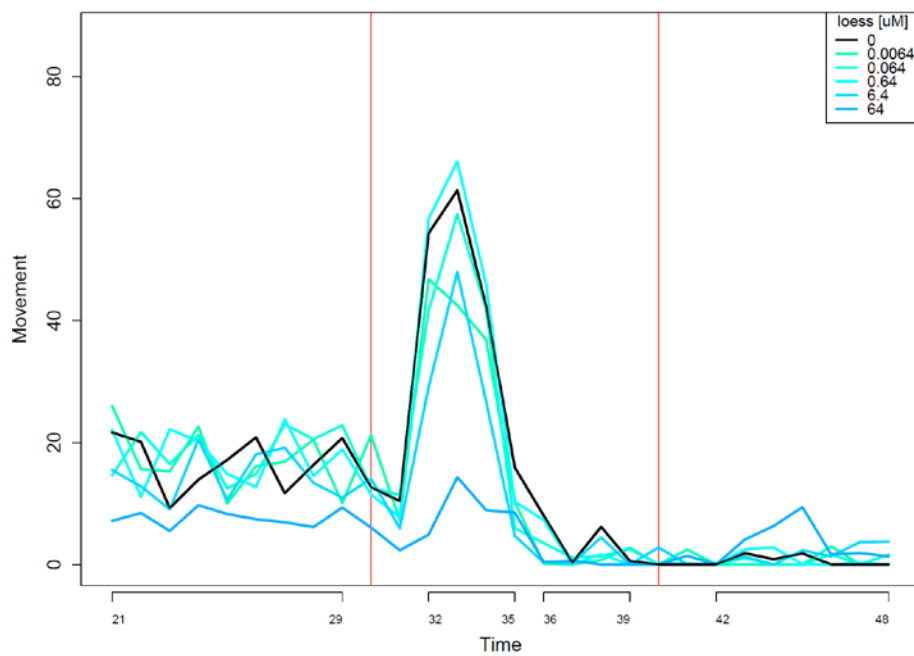
*mITP: Zebrafish larvae PMR assay results at 120 hpf*



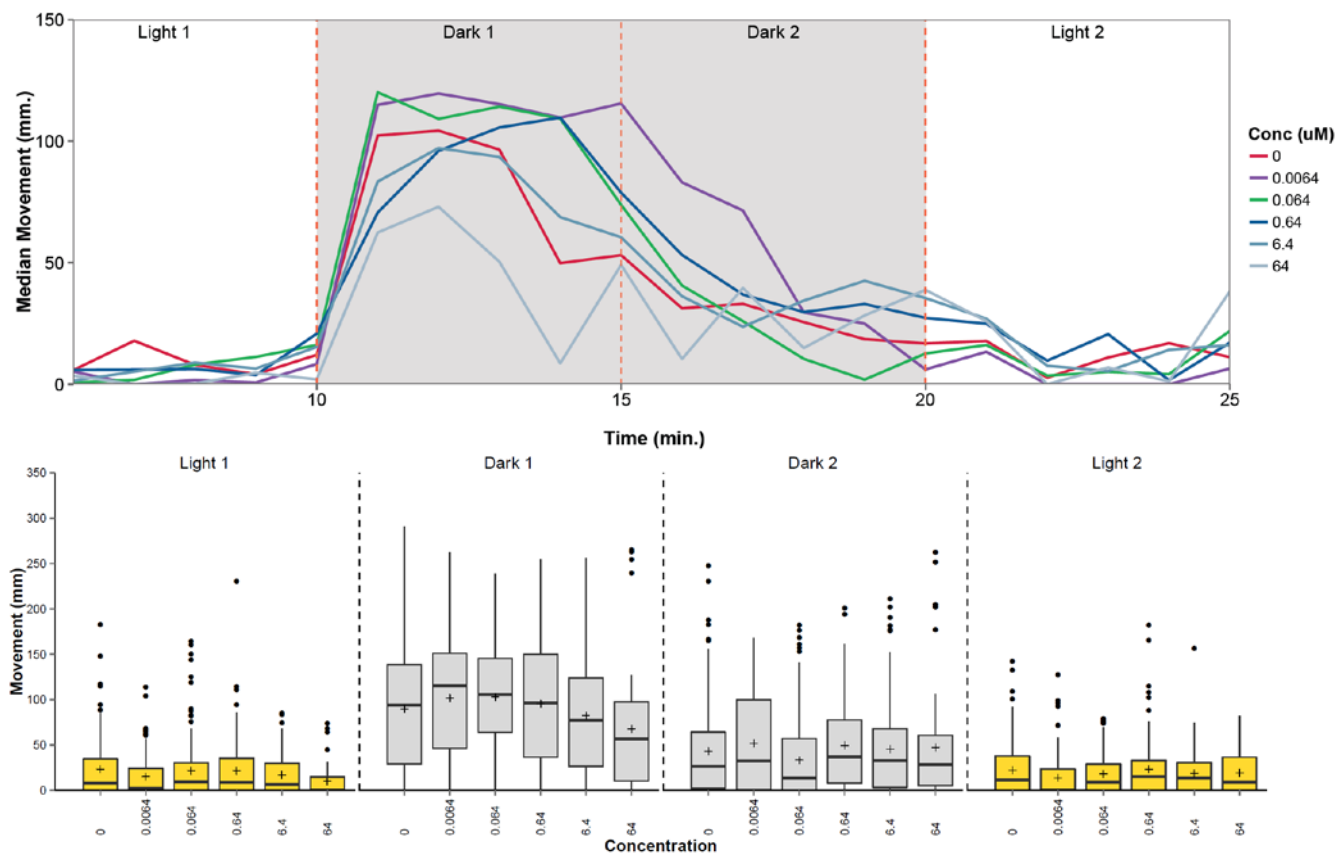
### ***o*-TCP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



### ***o-TCP: Zebrafish embryo PRAT results at 24 hpf***



*o*-TCP: Zebrafish larvae PMR assay results at 120 hpf

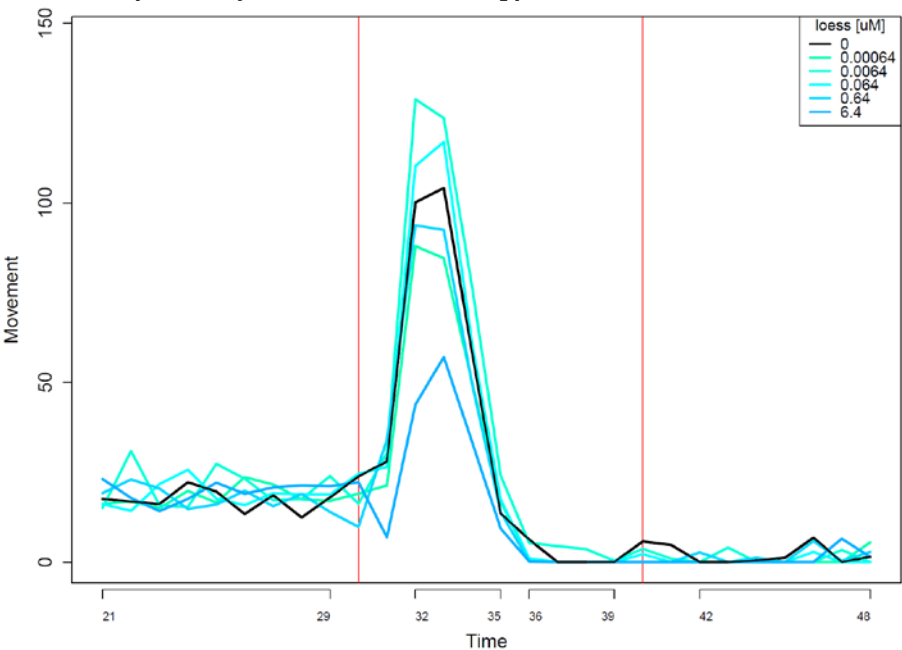


**Resorcinol A bis-(diphenyl phosphate) (RDP)**

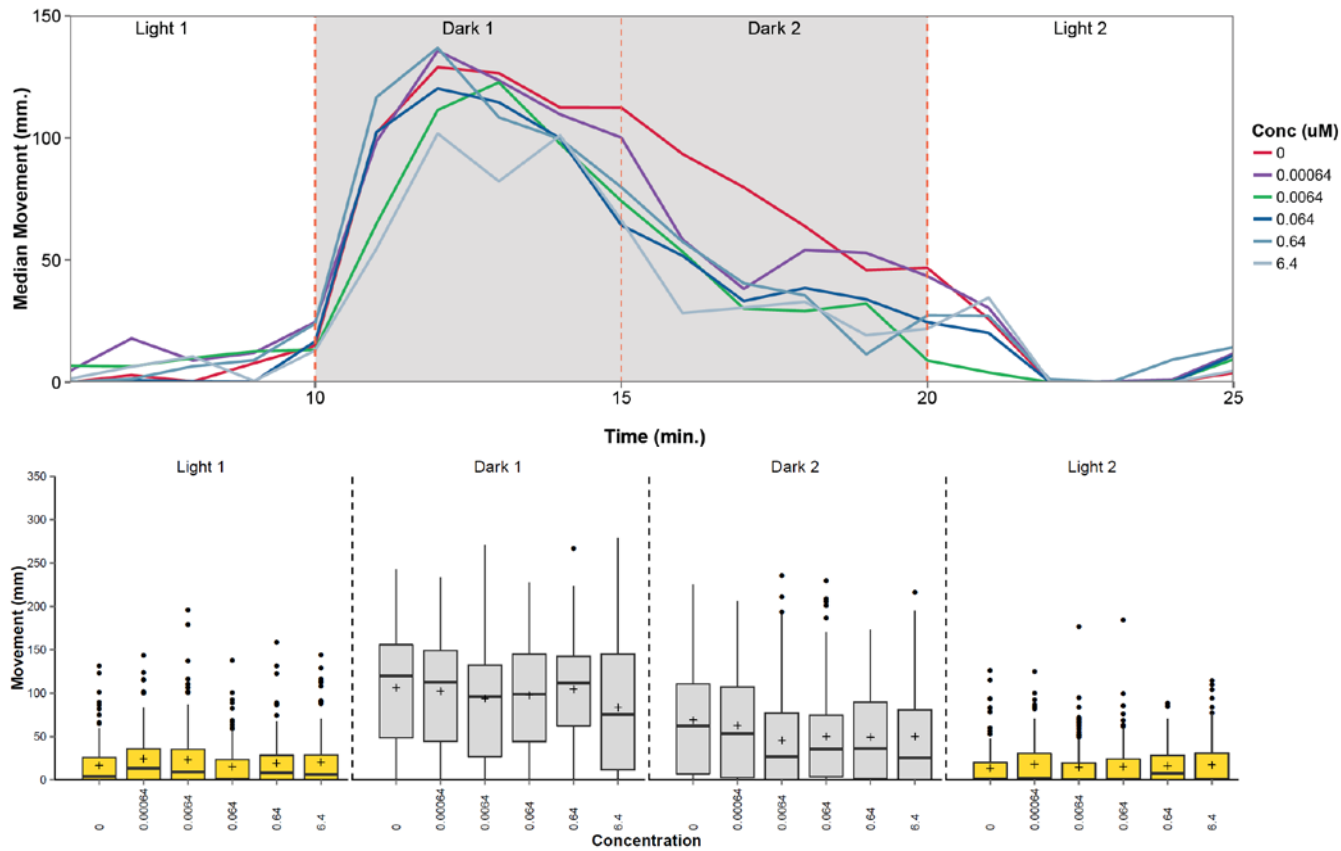
***RDP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***RDP: Zebrafish embryo PRAT results at 24 hpf***



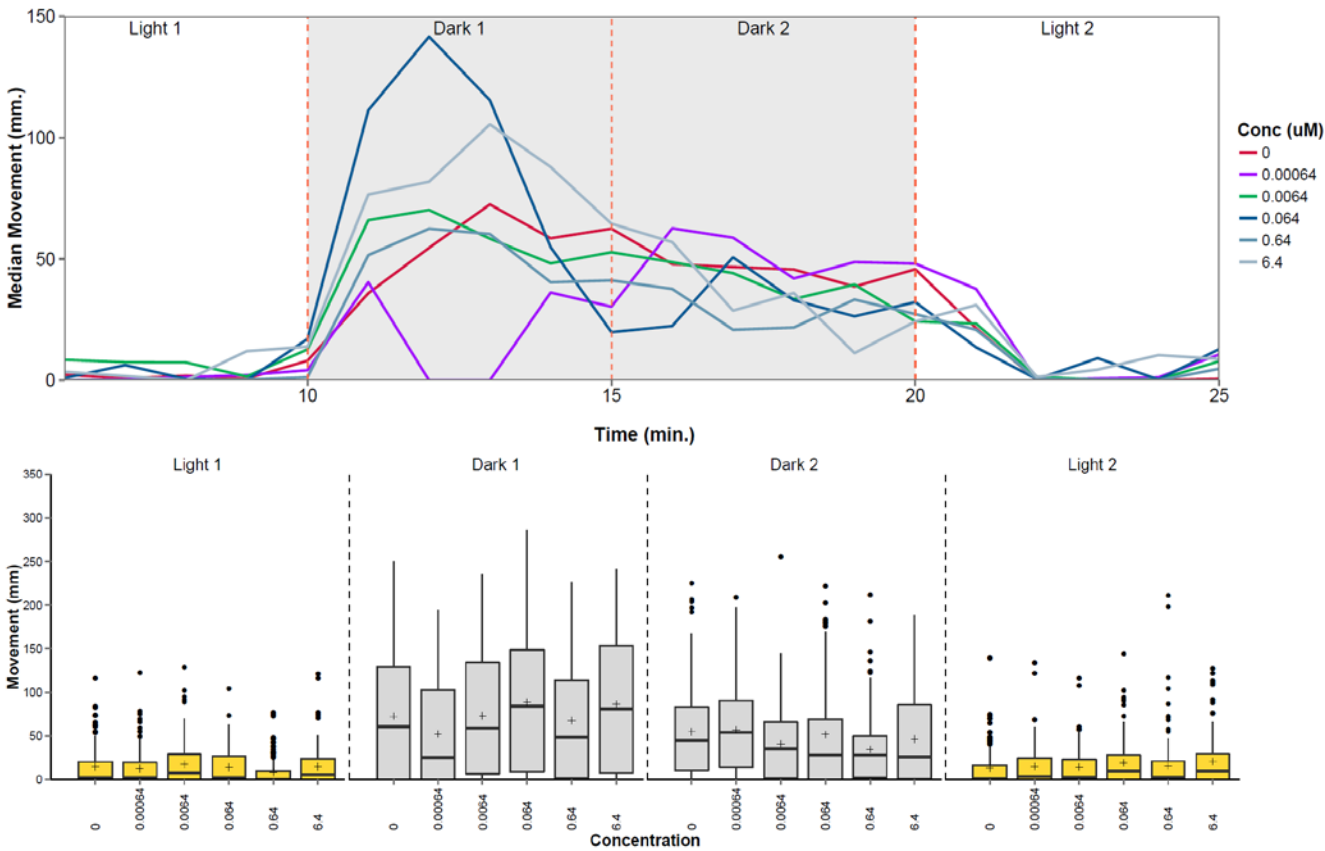
RDP: Zebrafish larvae PMR assay results at 120 hpf



### ***TBP: Zebrafish Mortality and Malformations at 24 and 120 hpf***

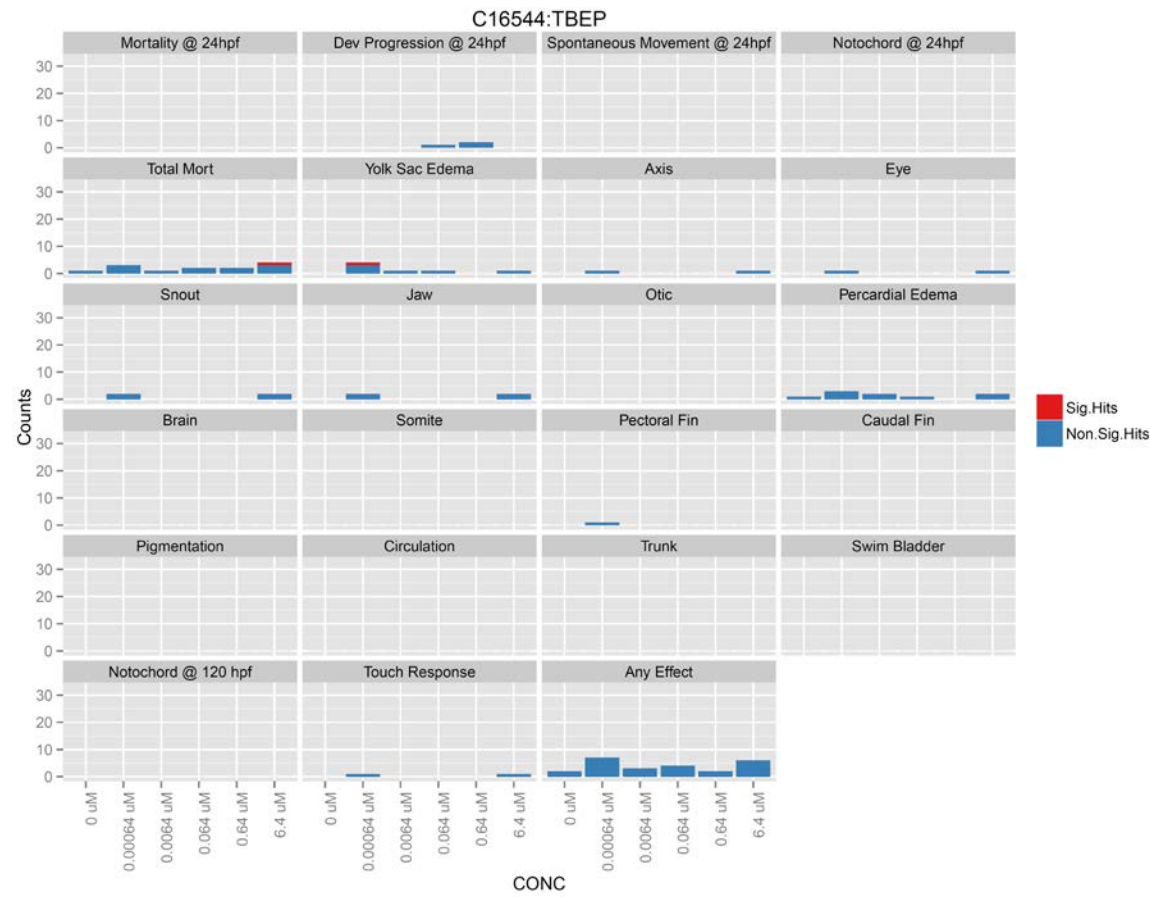


TBP: Zebrafish larvae PMR assay results at 120 hpf

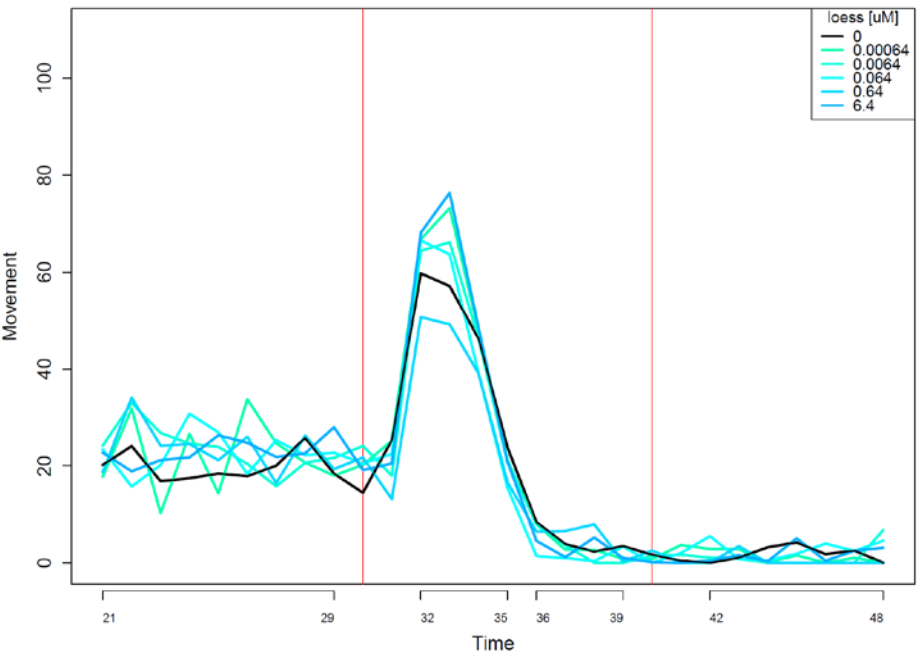


**Tris (2-butoxyethyl) phosphate TBEP**

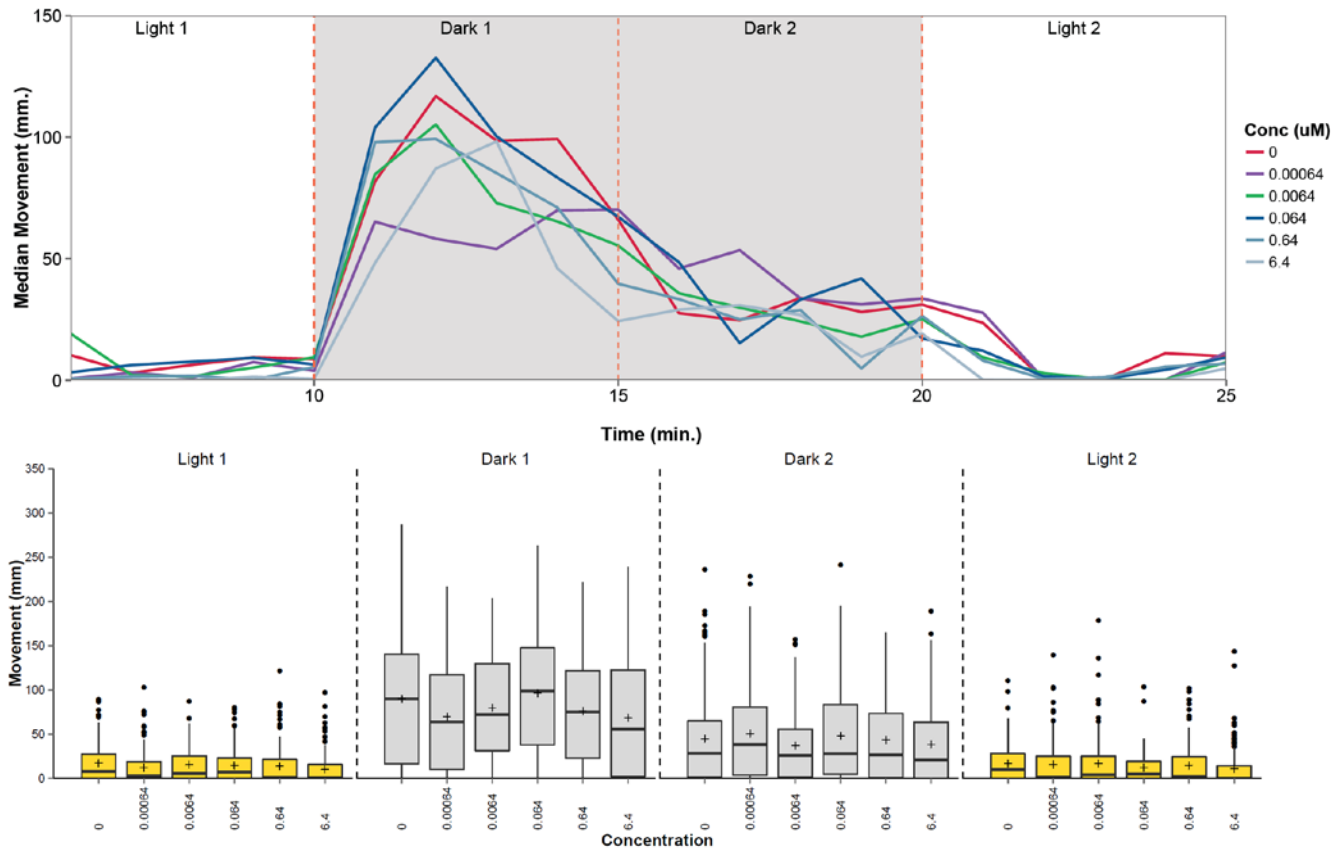
**TBEP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TBEP: Zebrafish embryo PRAT results at 24 hpf**

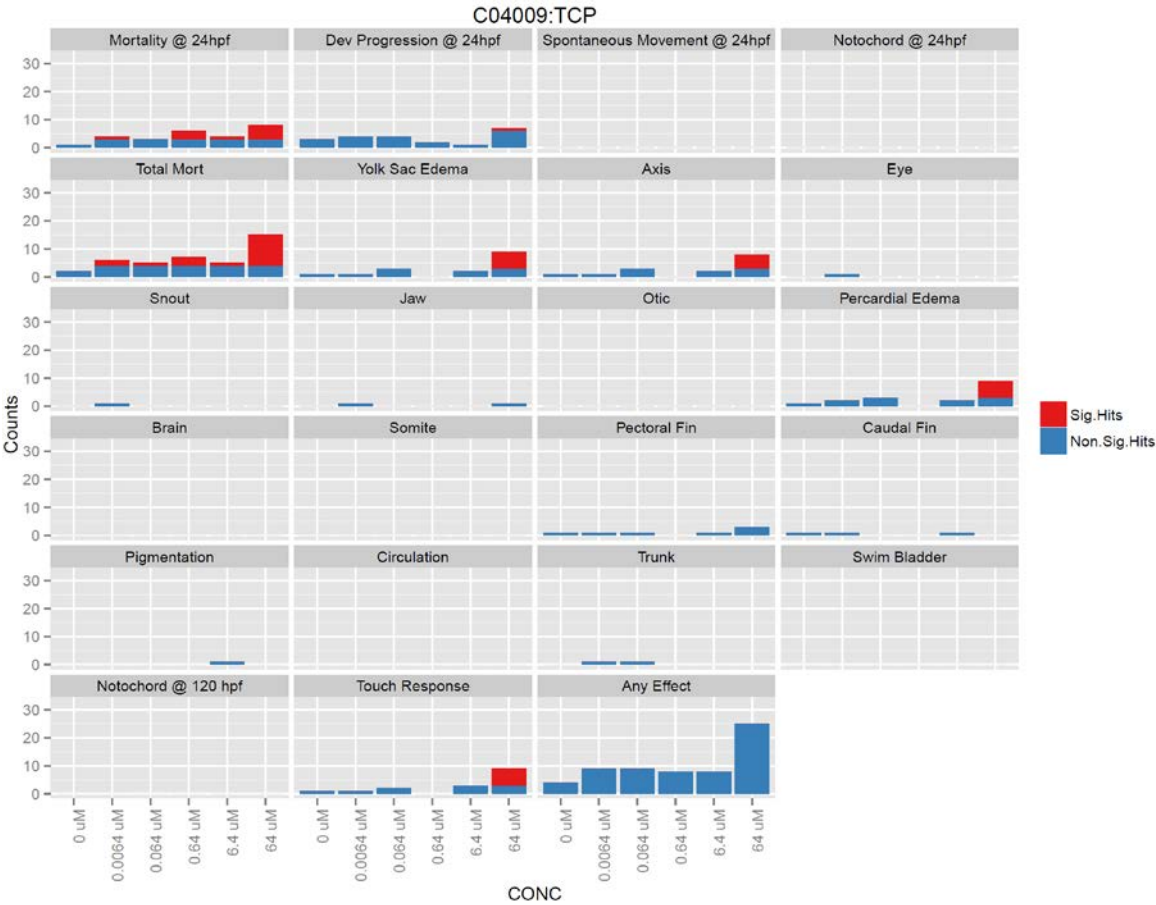


**TBEP: Zebrafish larvae PMR assay results at 120 hpf**

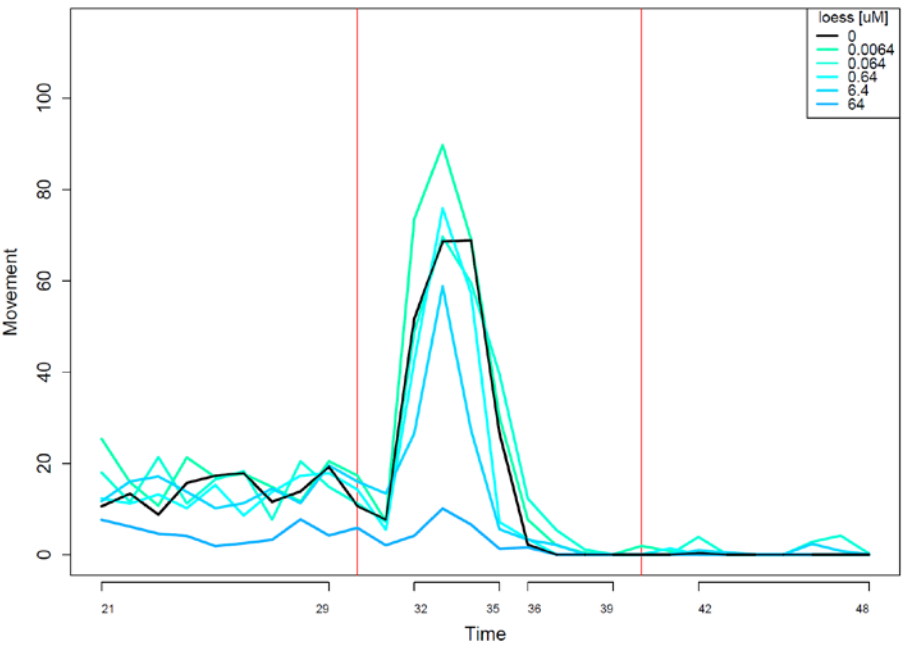


**Tricresyl phosphate (TCP)**

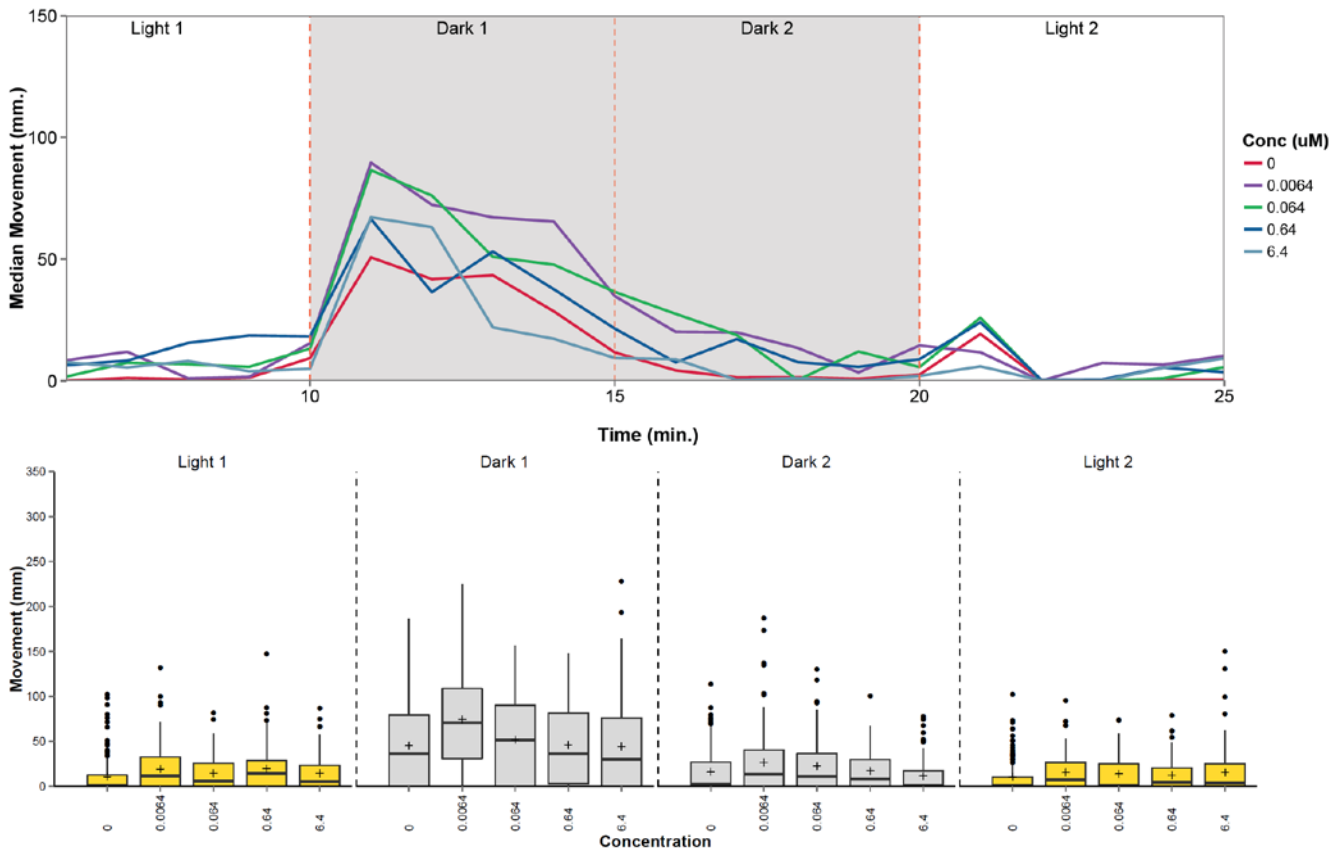
**TCP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TCP: Zebrafish embryo PRAT results at 24 hpf**

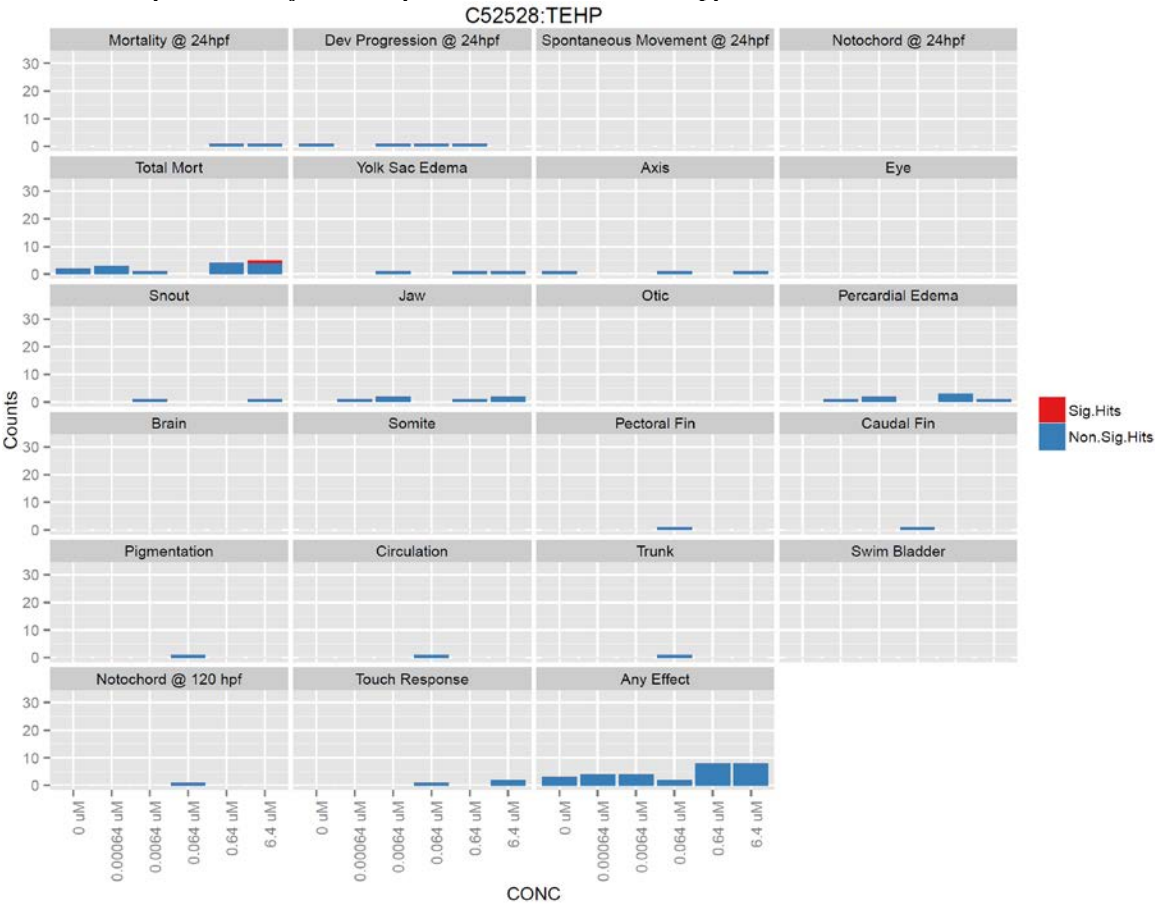


TCP: Zebrafish larvae PMR assay results at 120 hpf

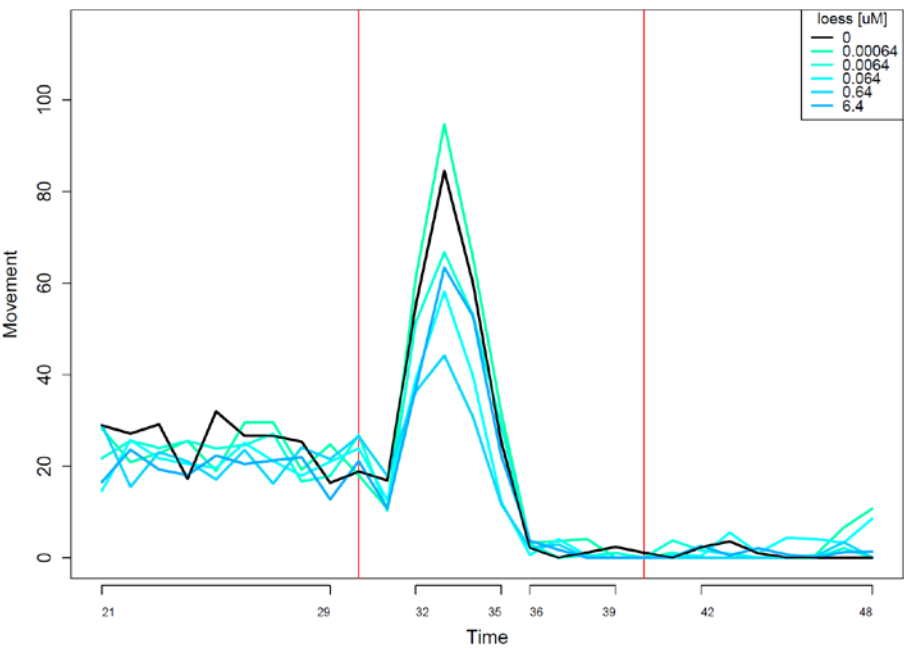


**Tris (2-ethylhexyl) phosphate (TEHP)**

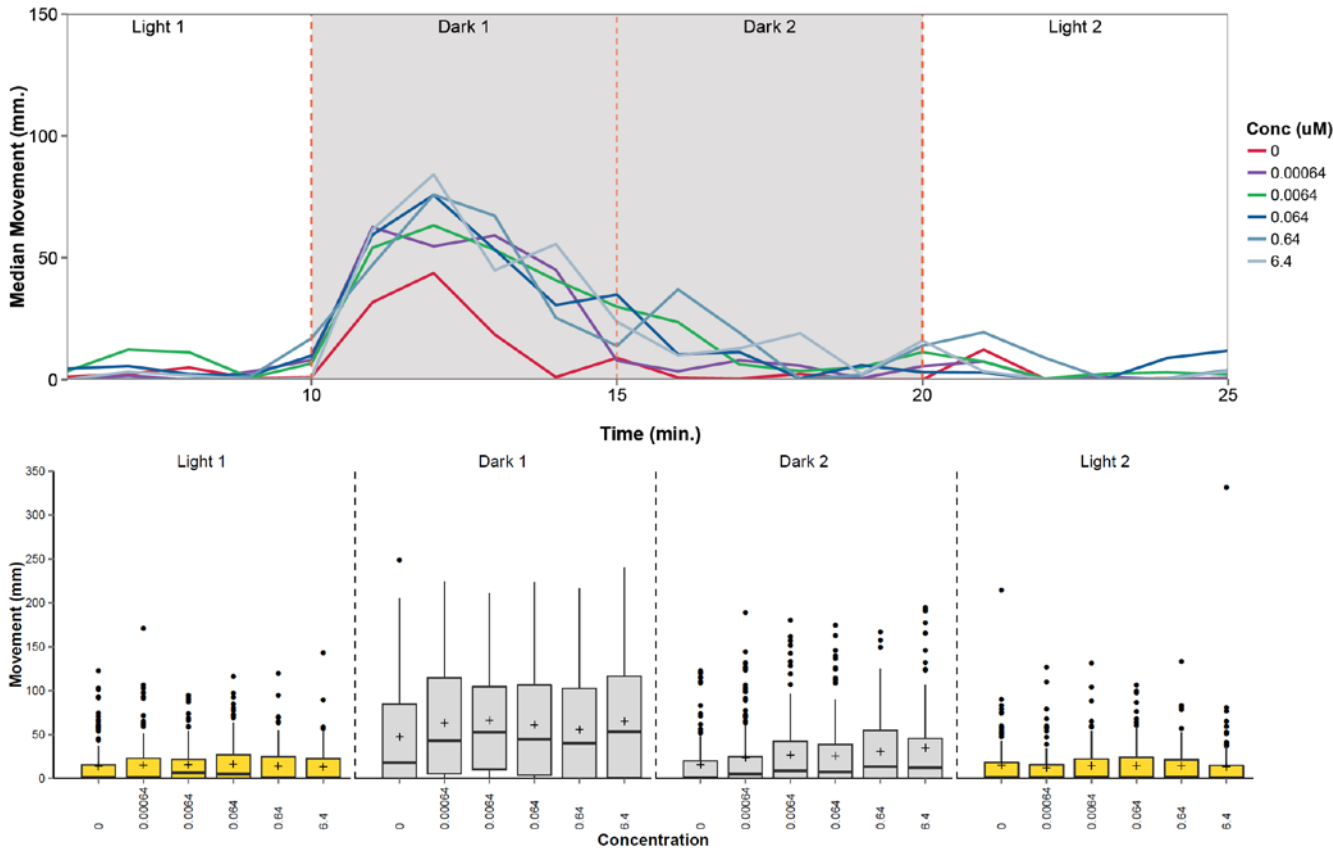
**TEHP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TEHP: Zebrafish embryo PRAT results at 24 hpf**

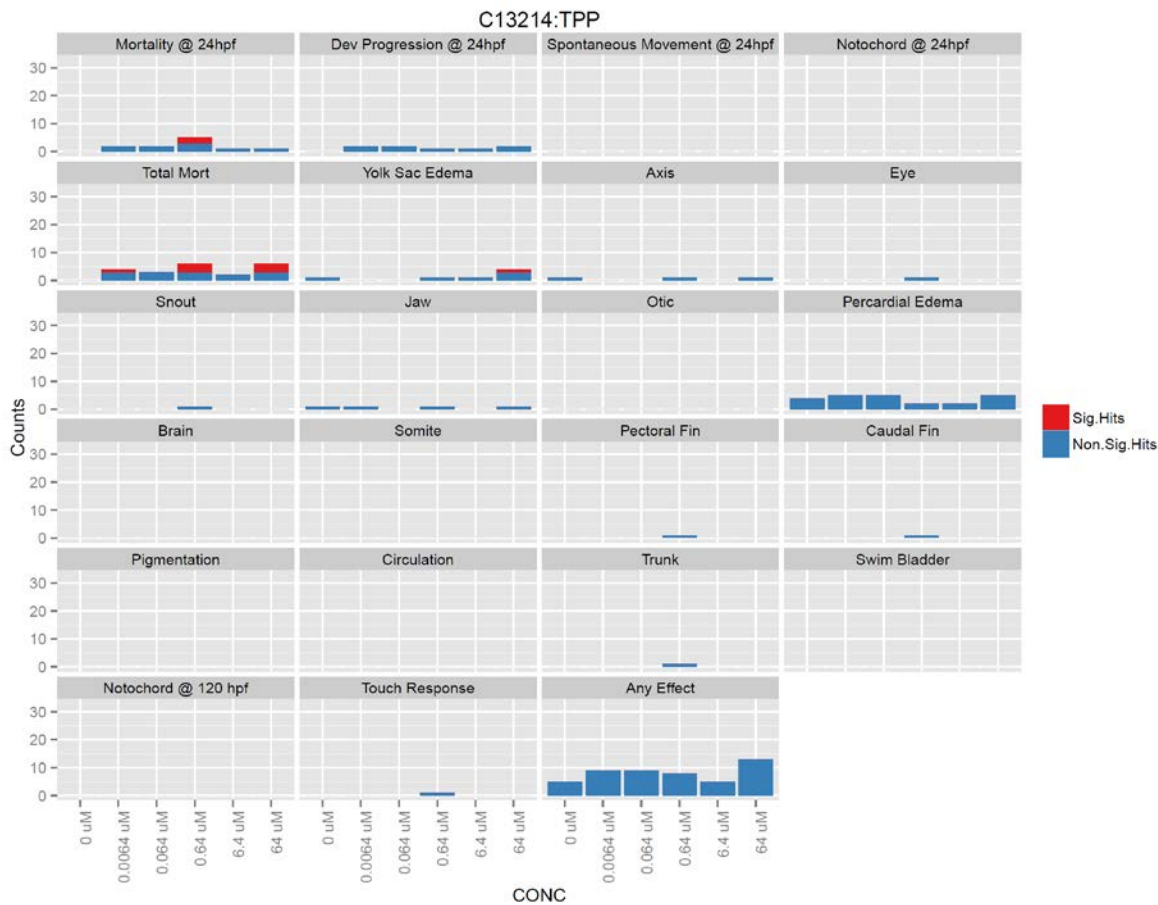


TEHP: Zebrafish larvae PMR assay results at 120 hpf

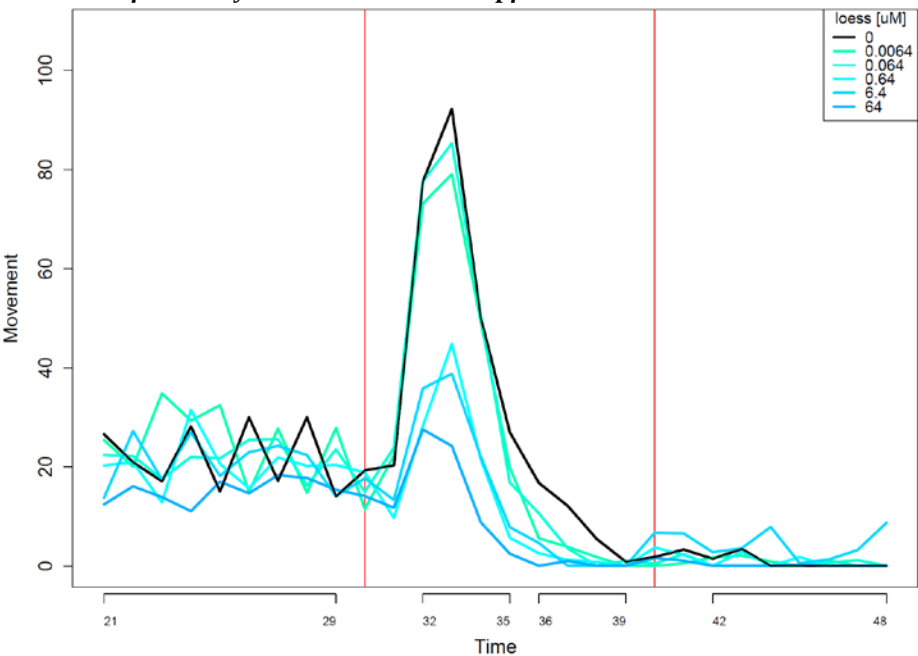


**Triphenyl phosphate (TPP)**

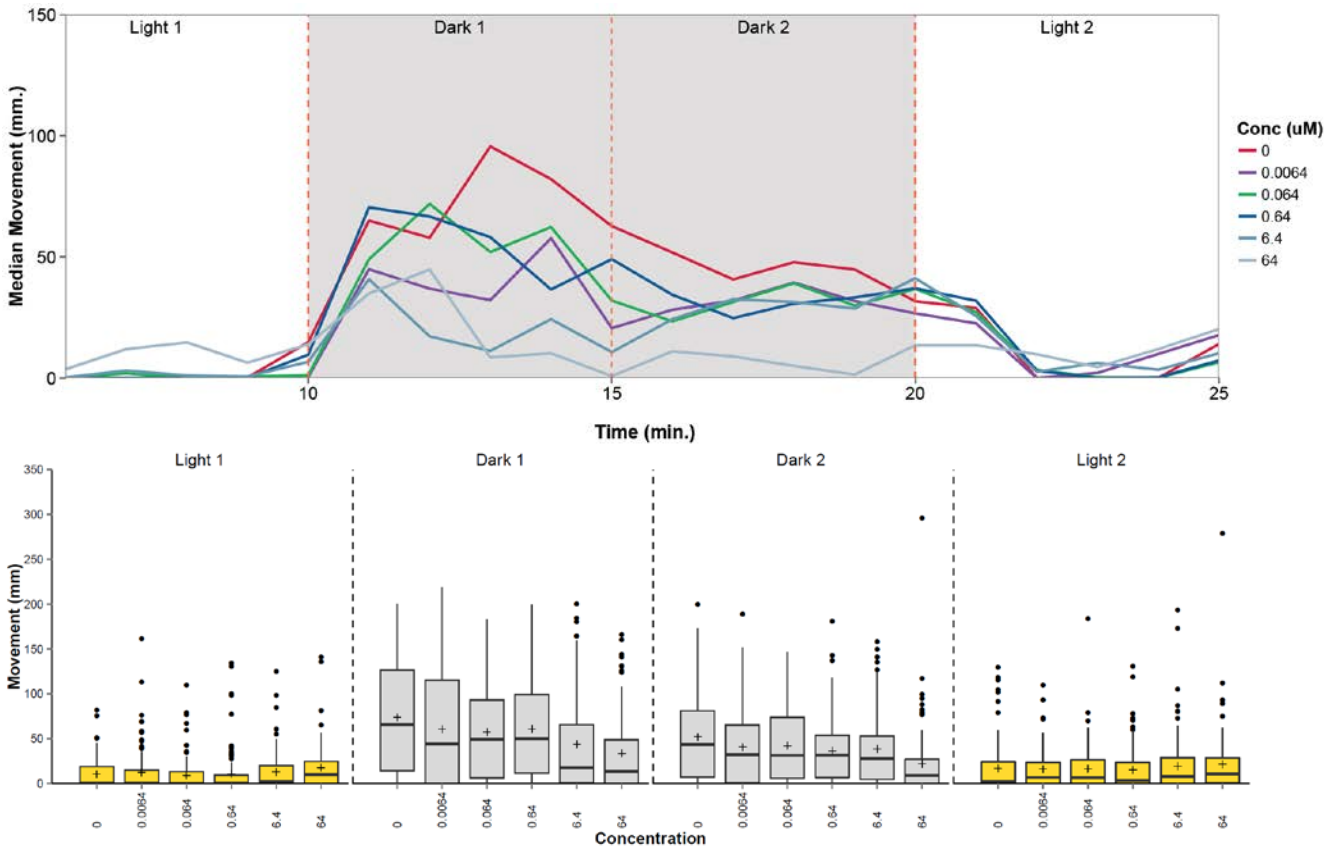
***TPP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***TPP: Zebrafish embryo PRAT results at 24 hpf***

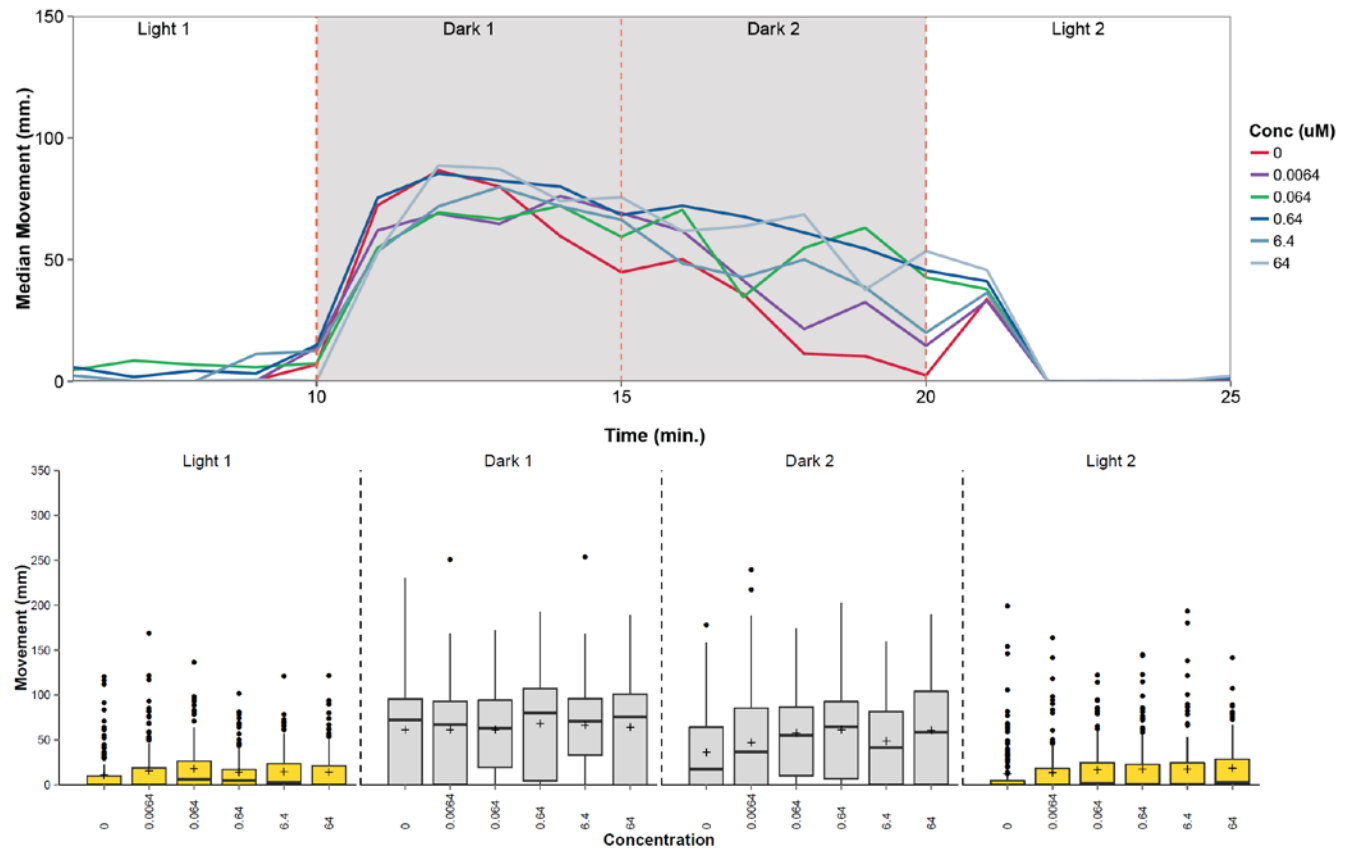


TPP: Zebrafish larvae PMR assay results at 120 hpf



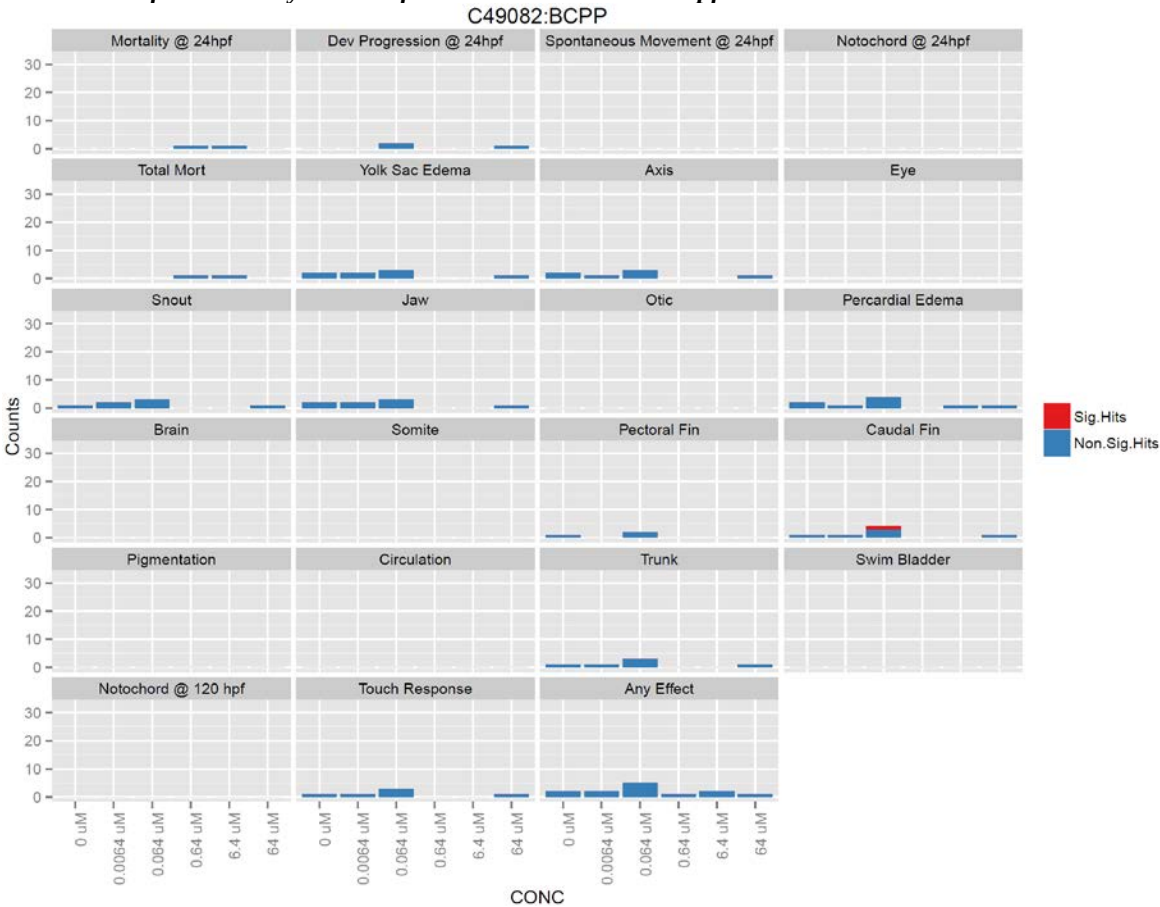


**BCPCP: Zebrafish larvae PMR assay results at 120 hpf**

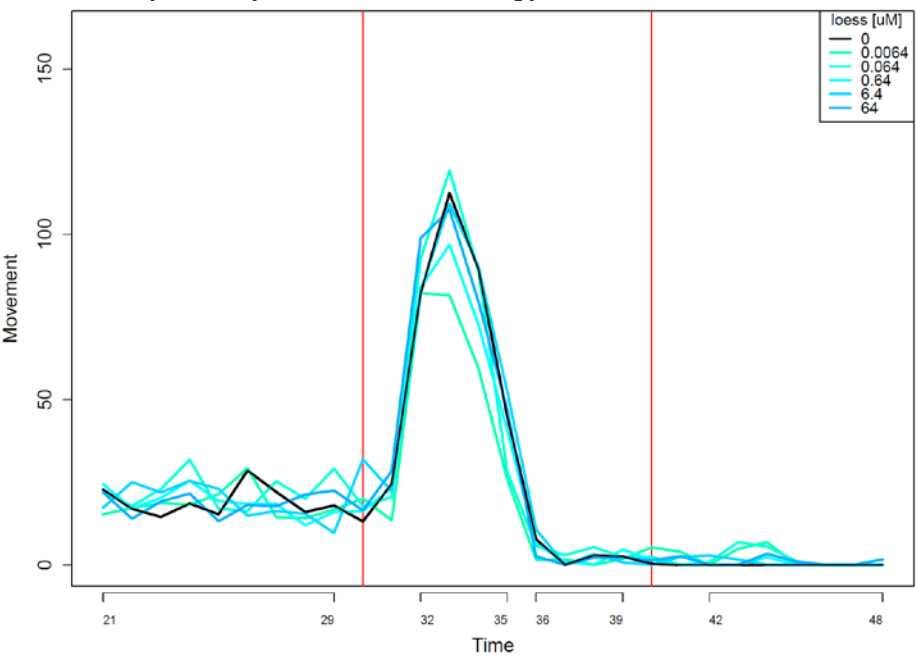


**Bis (2-chloropropyl) phosphate (BCPP)**

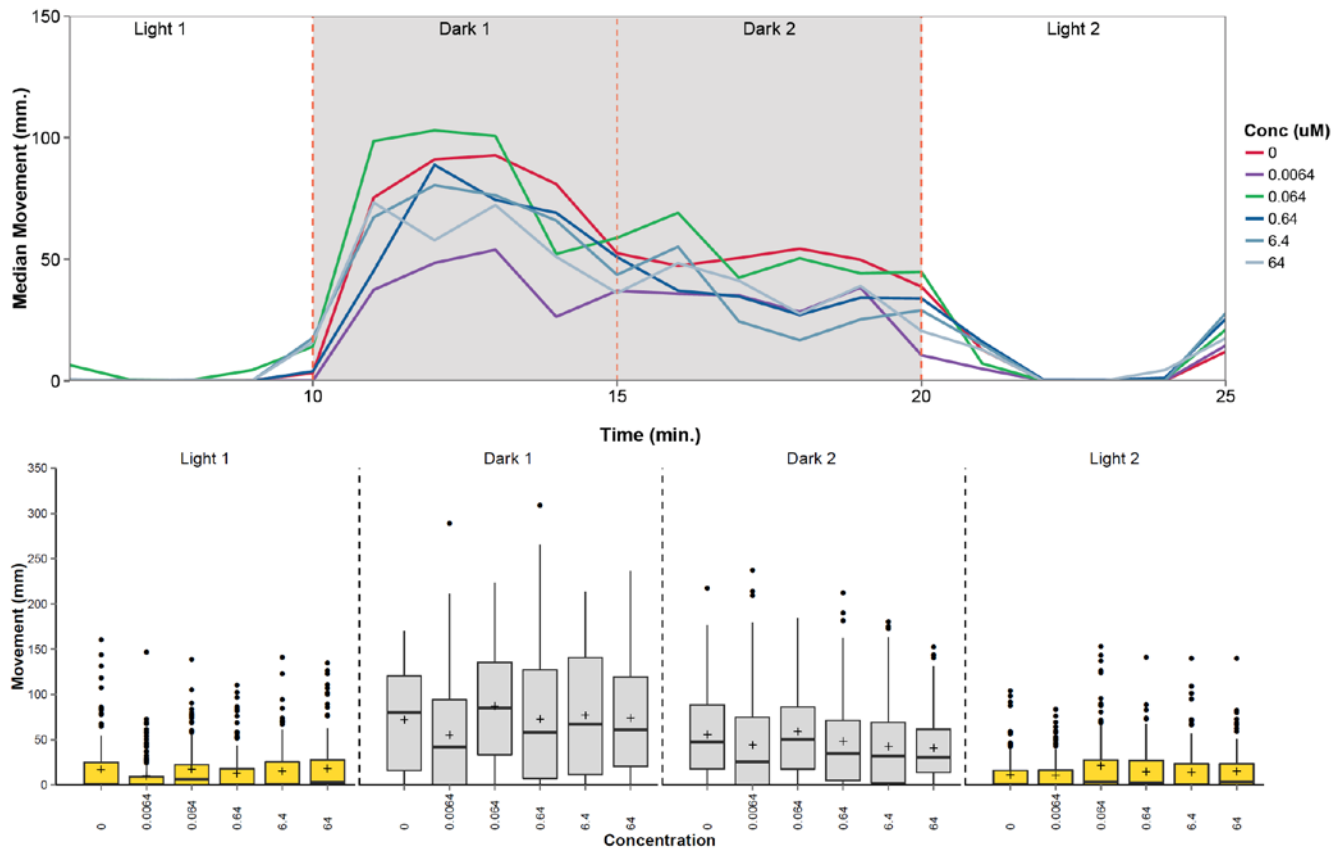
***BCPP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***BCPP: Zebrafish embryo PRAT results at 24 hpf***



BCPP: Zebrafish larvae PMR assay results at 120 hpf

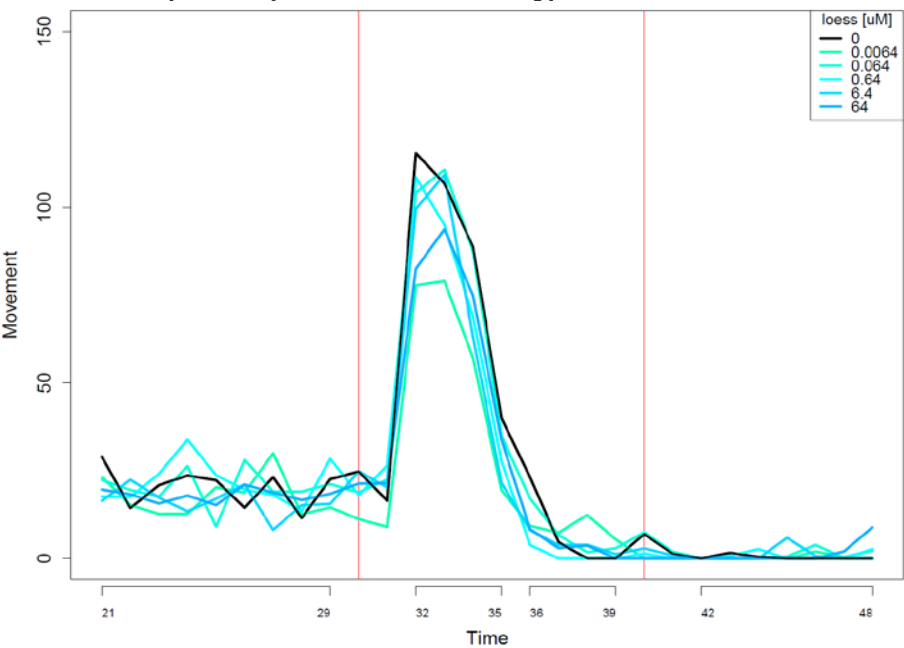


**Bis(1,3-dichloro-2-propyl)phosphate (BDCPP)**

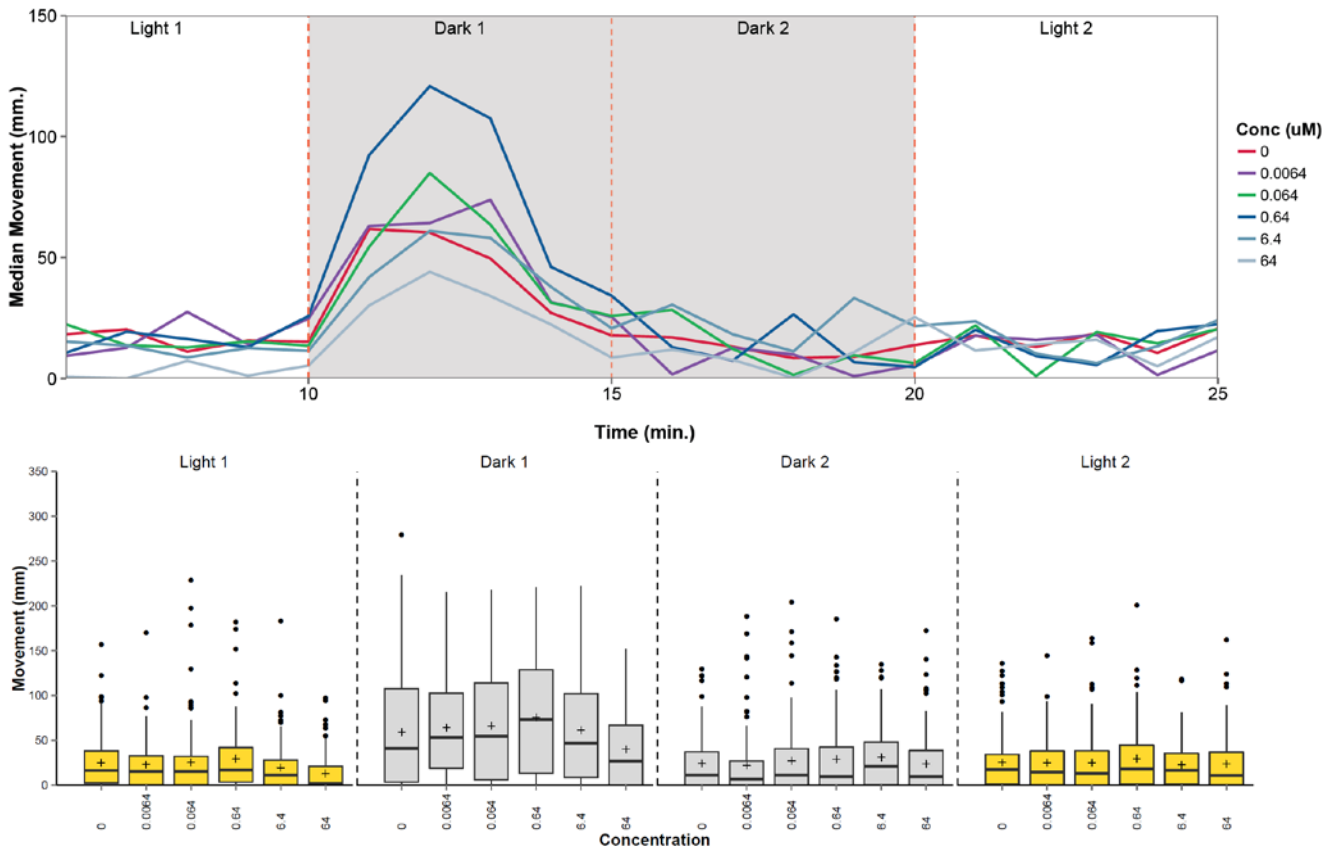
***BDCPP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***BDCPP: Zebrafish embryo PRAT results at 24 hpf***

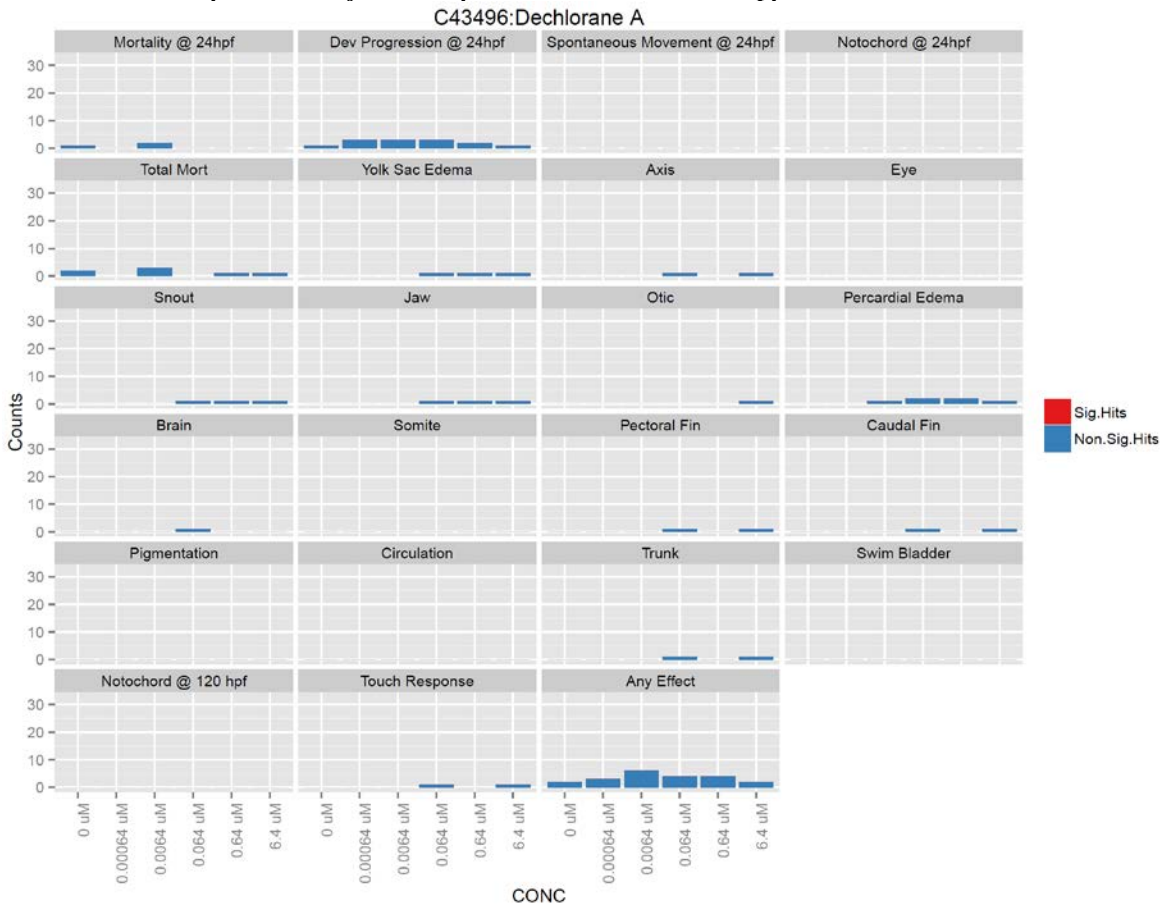


*BDCPP: Zebrafish larvae PMR assay results at 120 hpf*

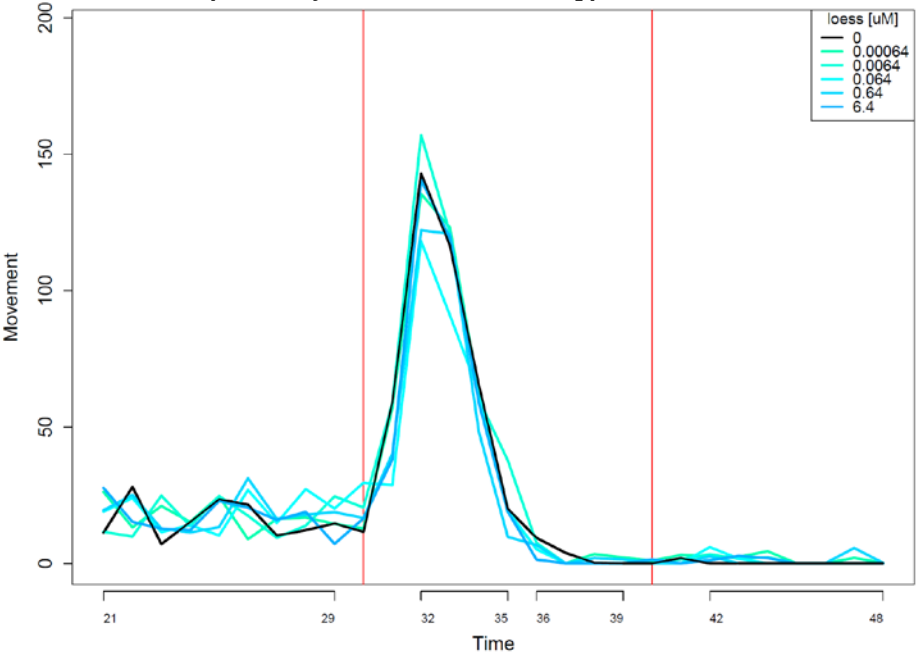


**Dechlorane Plus**

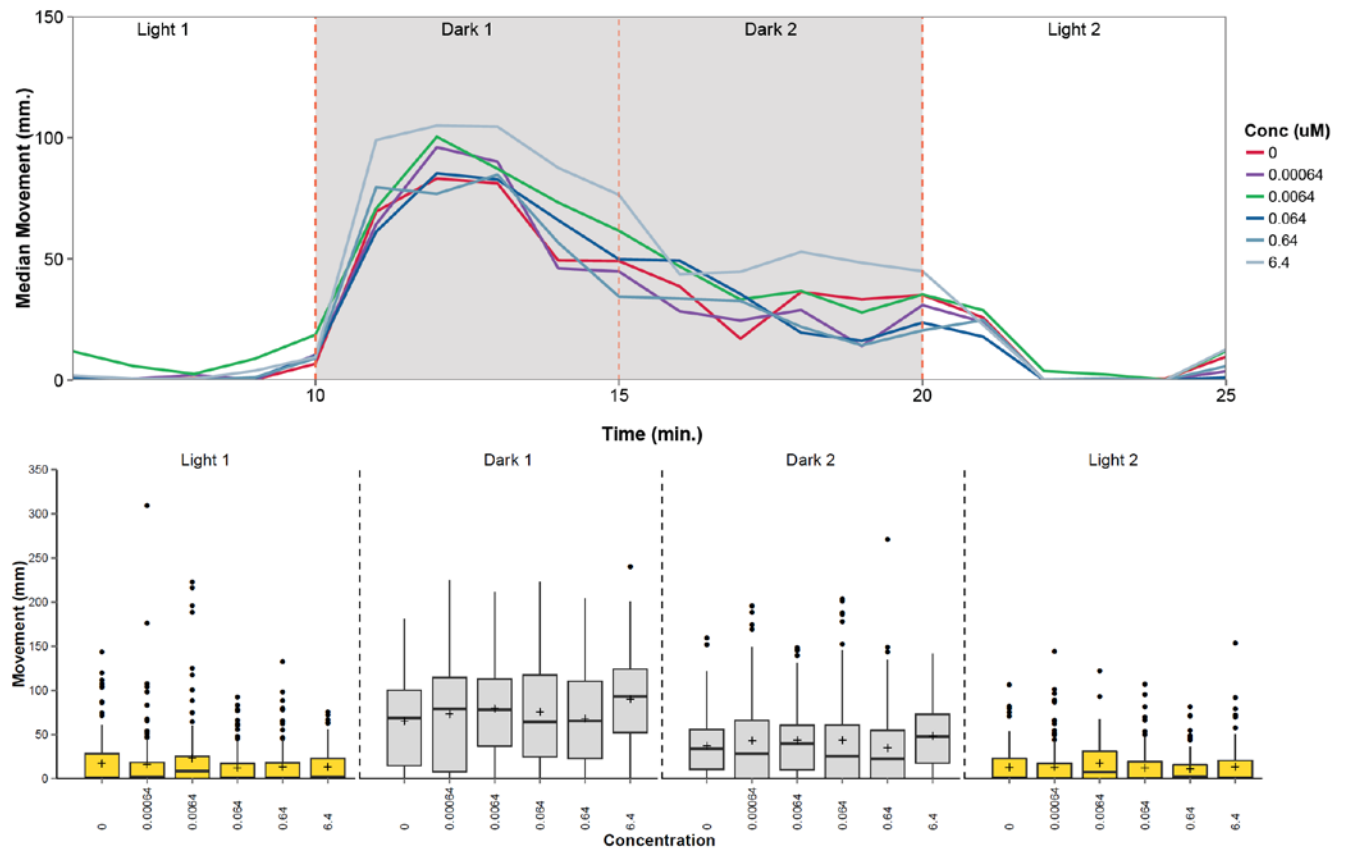
**Dechlorane: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**Dechlorane: Zebrafish embryo PRAT results at 24 hpf**

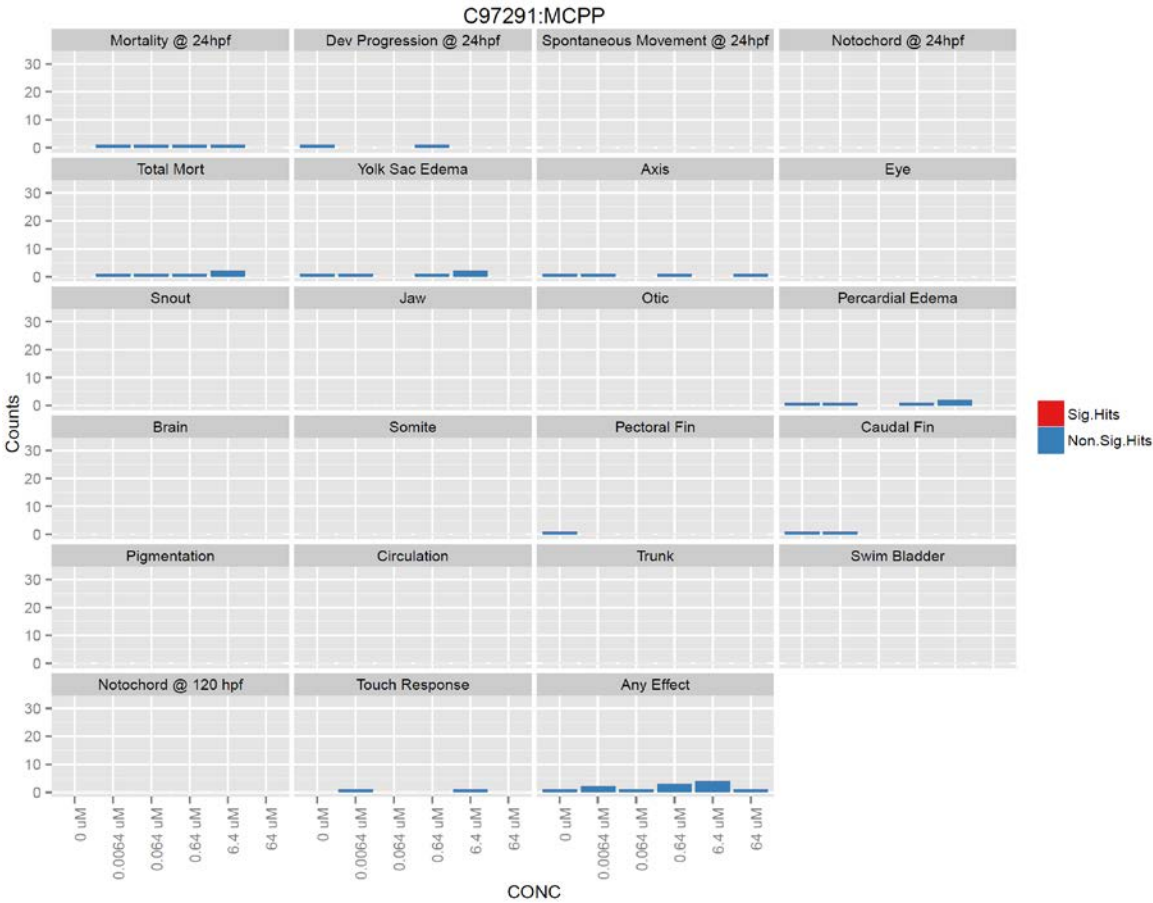


*Dechlorane: Zebrafish larvae PMR assay results at 120 hpf*

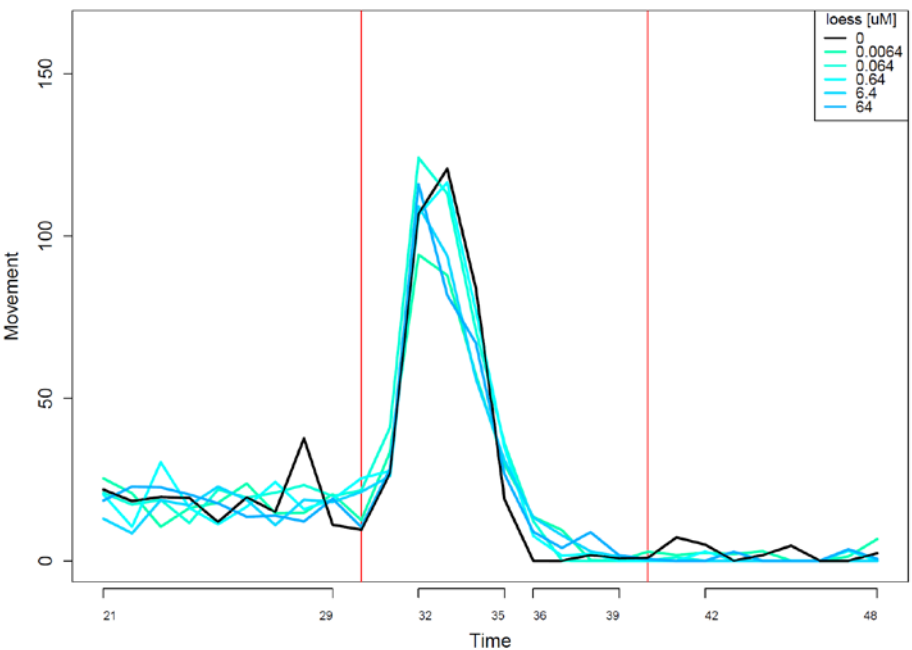


**Mono (2-chloropropyl) phosphate (MCPP)**

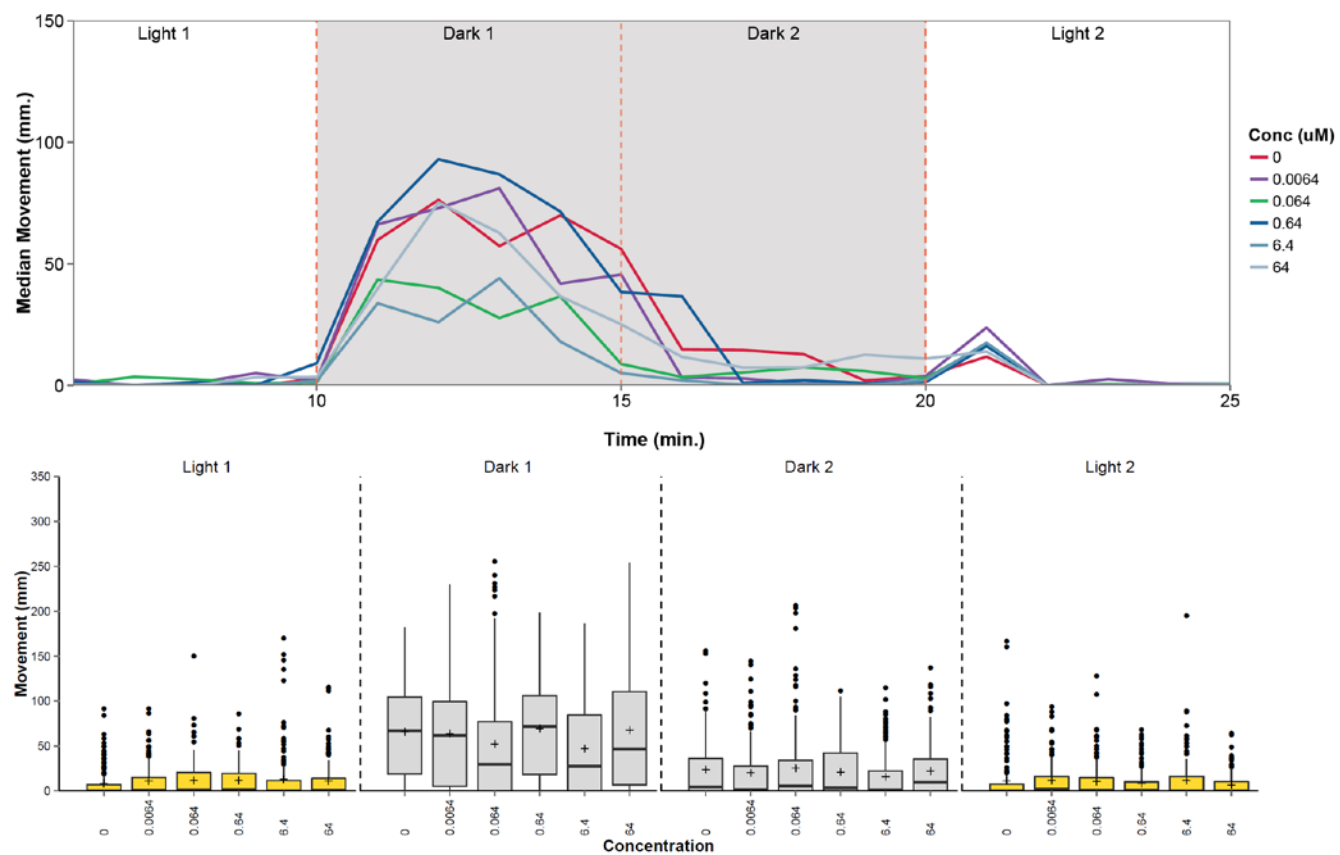
***MCPP: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***MCPP: Zebrafish embryo PRAT results at 24 hpf***

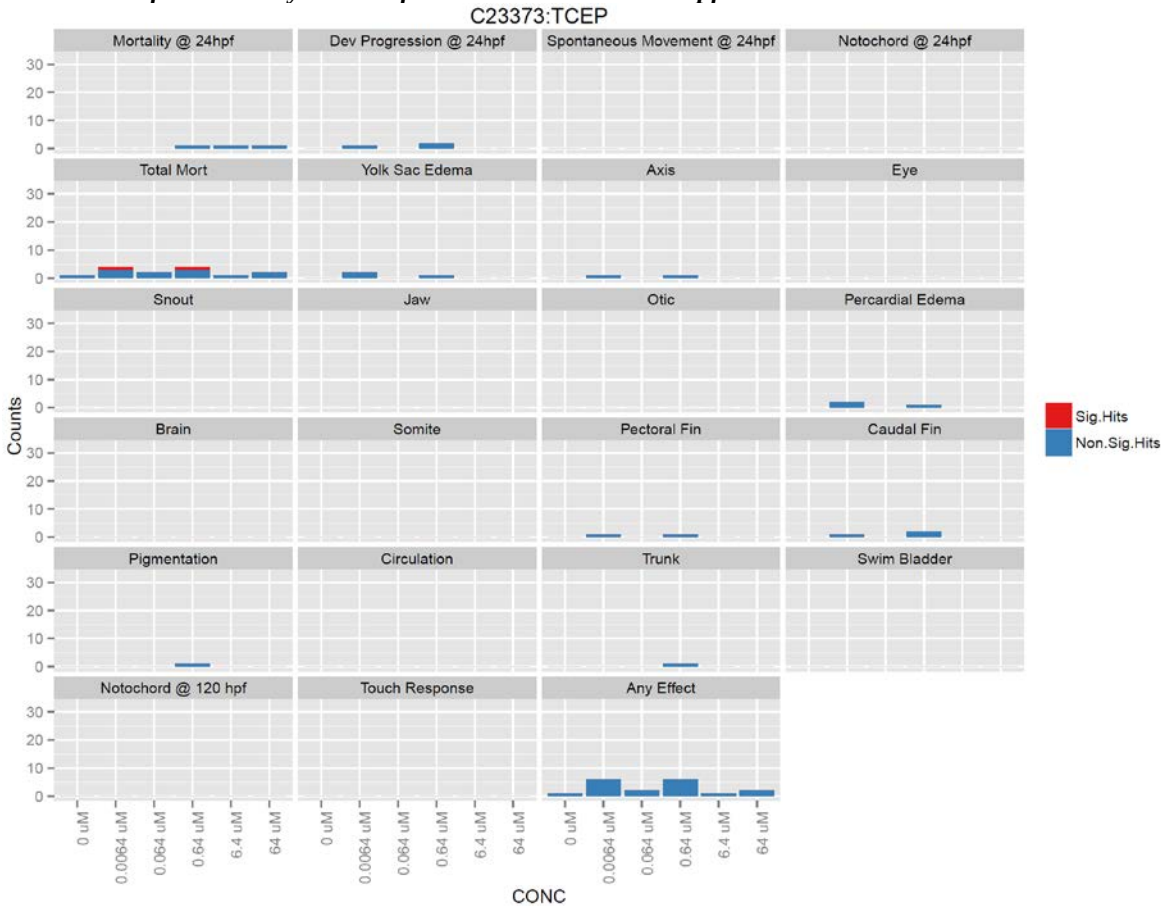


**MCPP: Zebrafish larvae PMR assay results at 120 hpf**

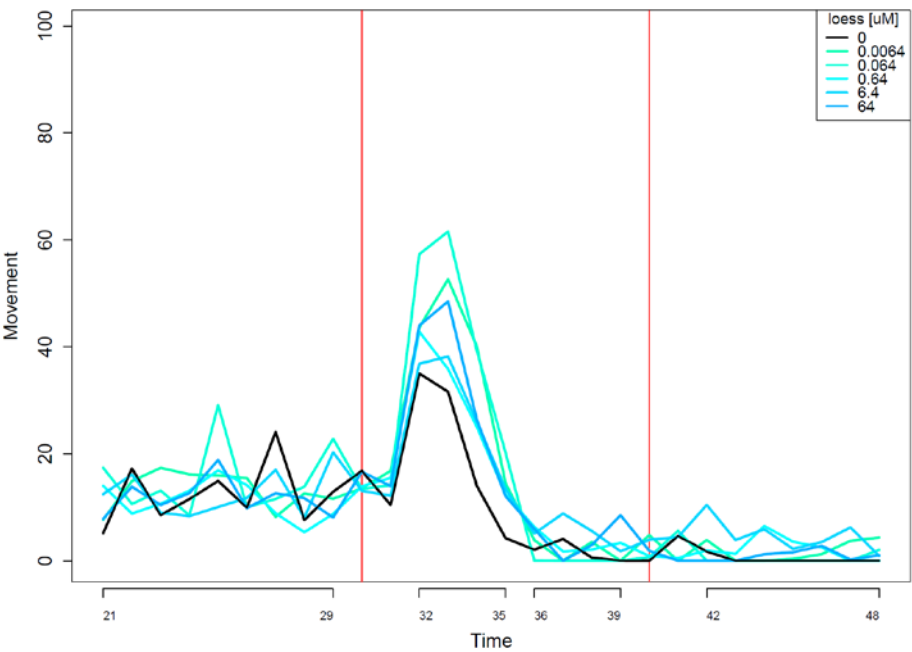


**Tris (2-chloroethyl) phosphate (TCEP)**

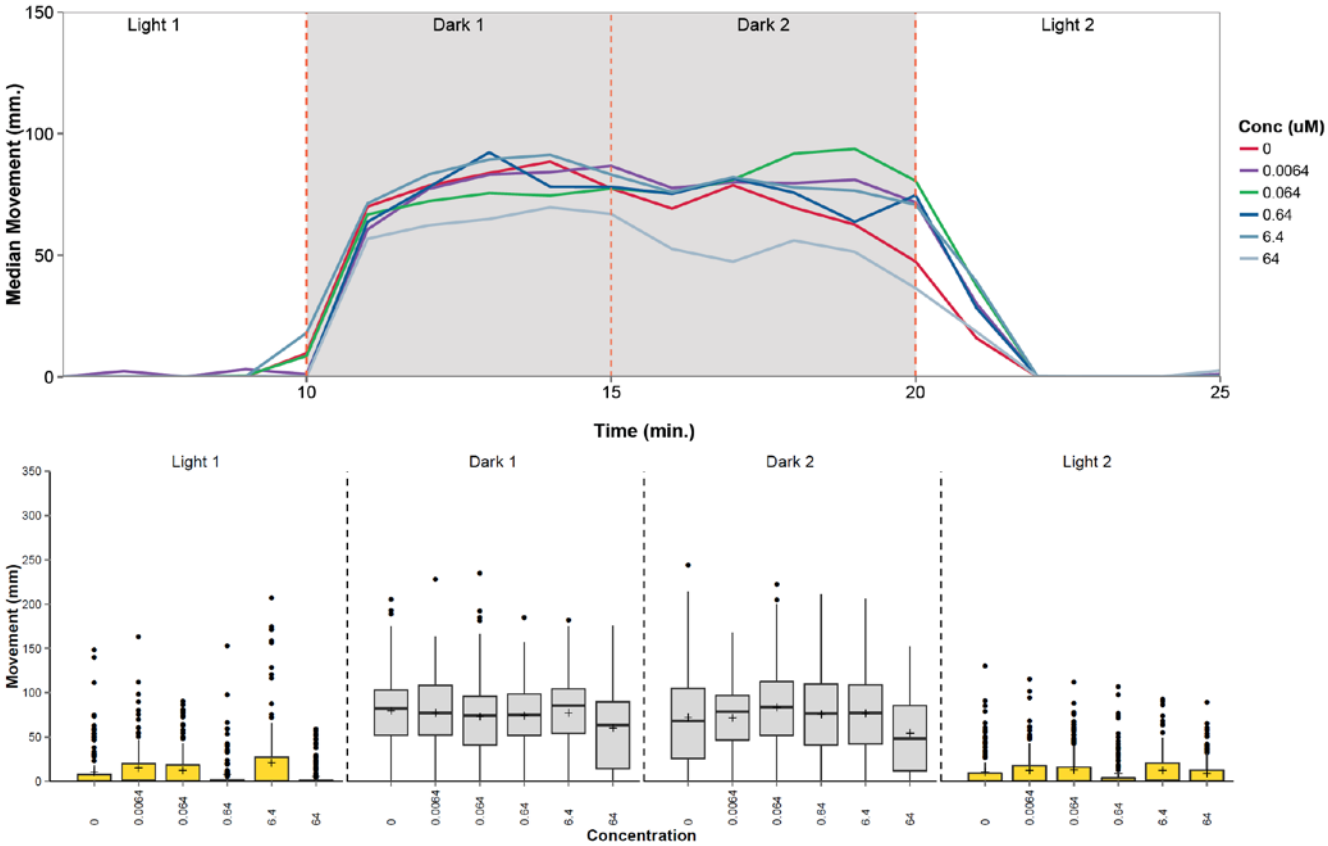
**TCEP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TCEP: Zebrafish embryo PRAT results at 24 hpf**

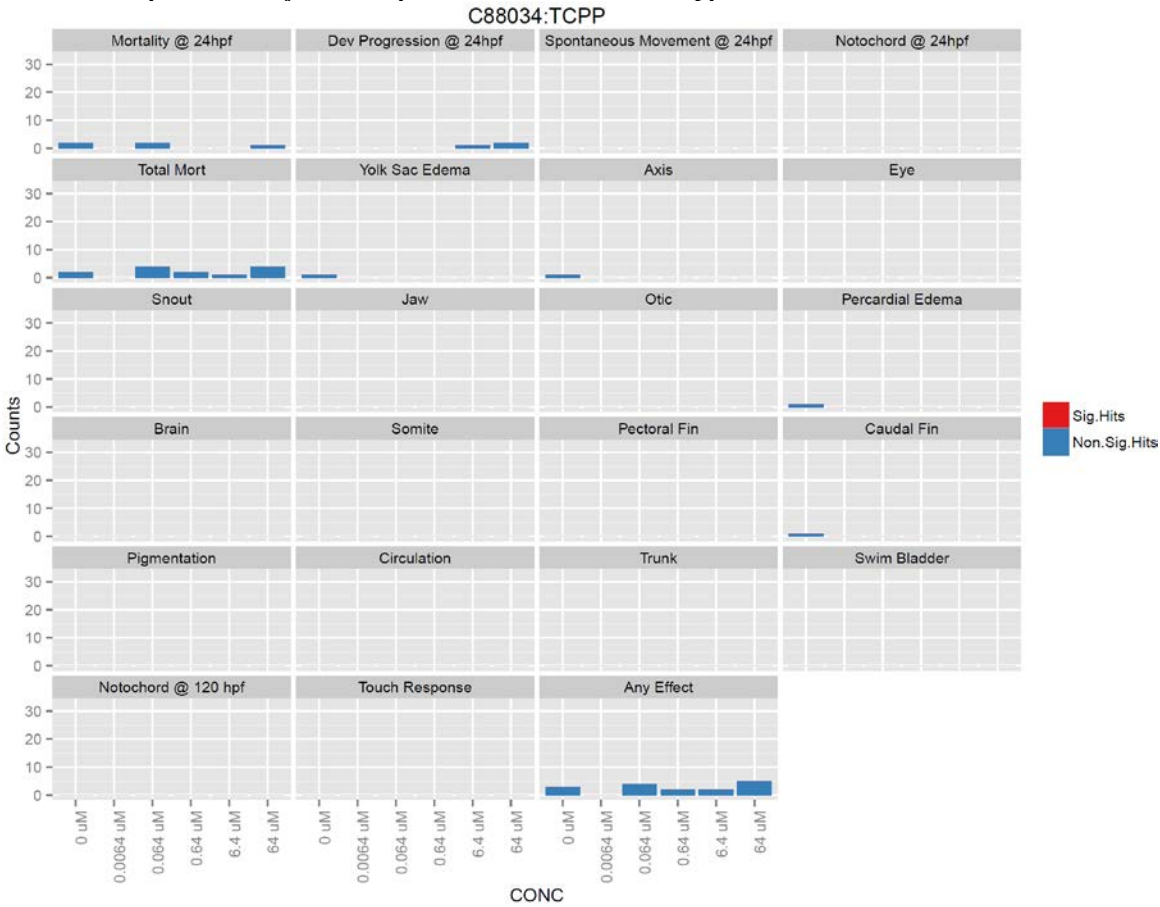


TCEP: Zebrafish larvae PMR assay results at 120 hpf

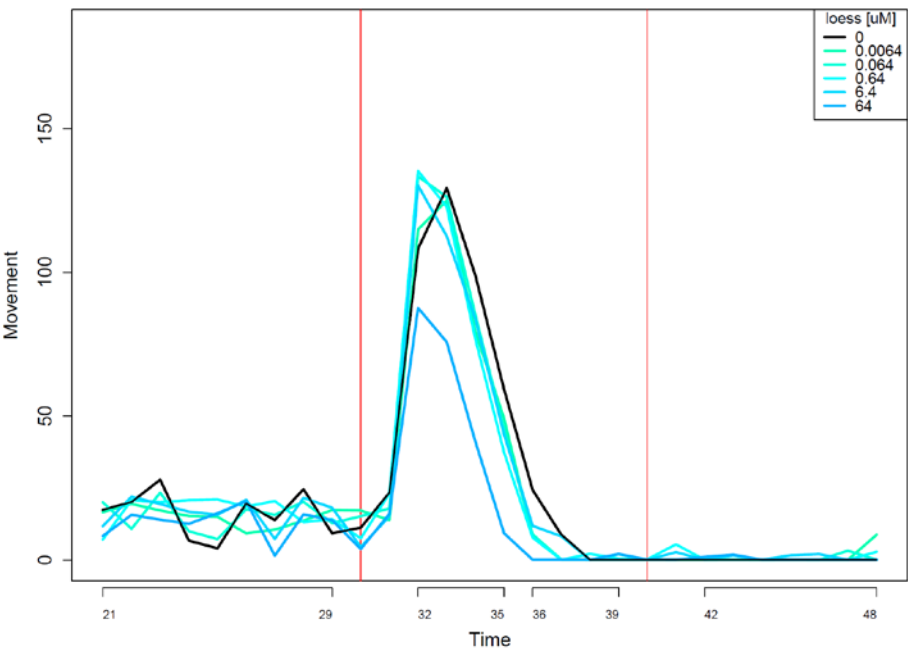


**Tris (1-chloropropyl) phosphate (TCPP)**

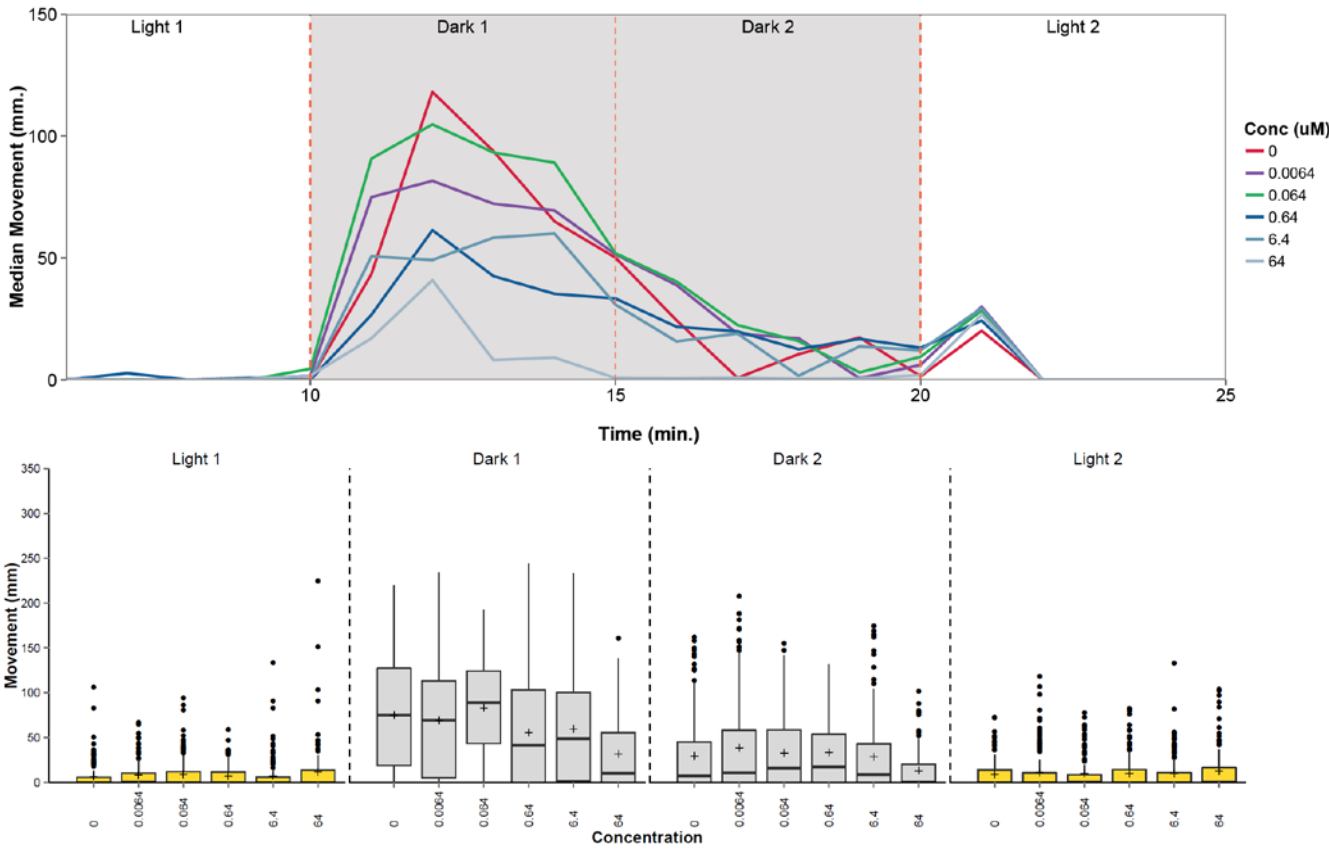
**TCPP: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TCPP: Zebrafish embryo PRAT results at 24 hpf**



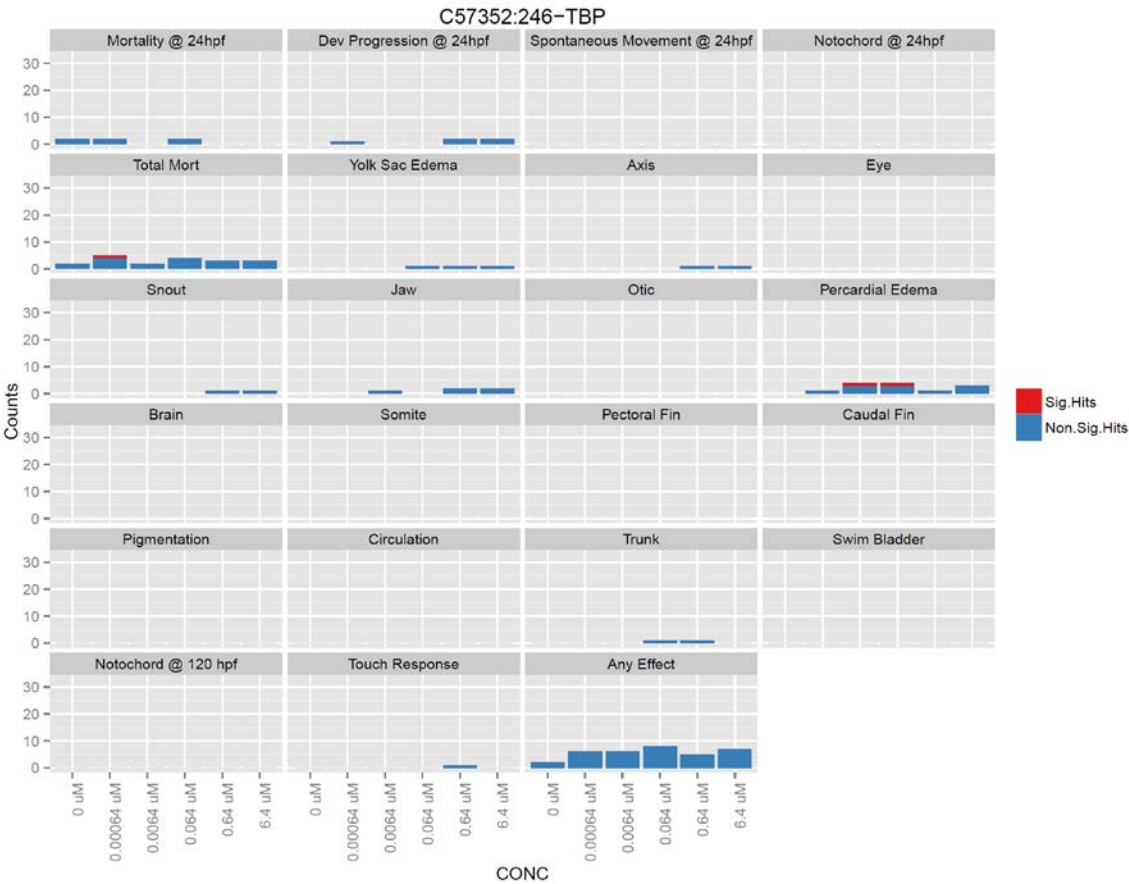
TCPP: Zebrafish larvae PMR assay results at 120 hpf



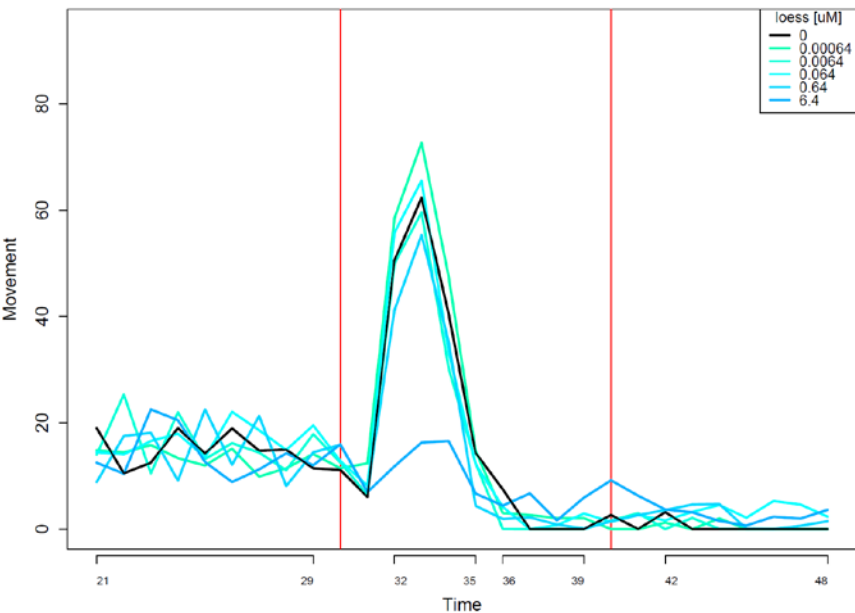
6. POLYBROMINATED DIPHENYL ETHER (PBDE) FLAME RETARDANTS

2,4,6-Tribromophenol (2,4,6-TBP)

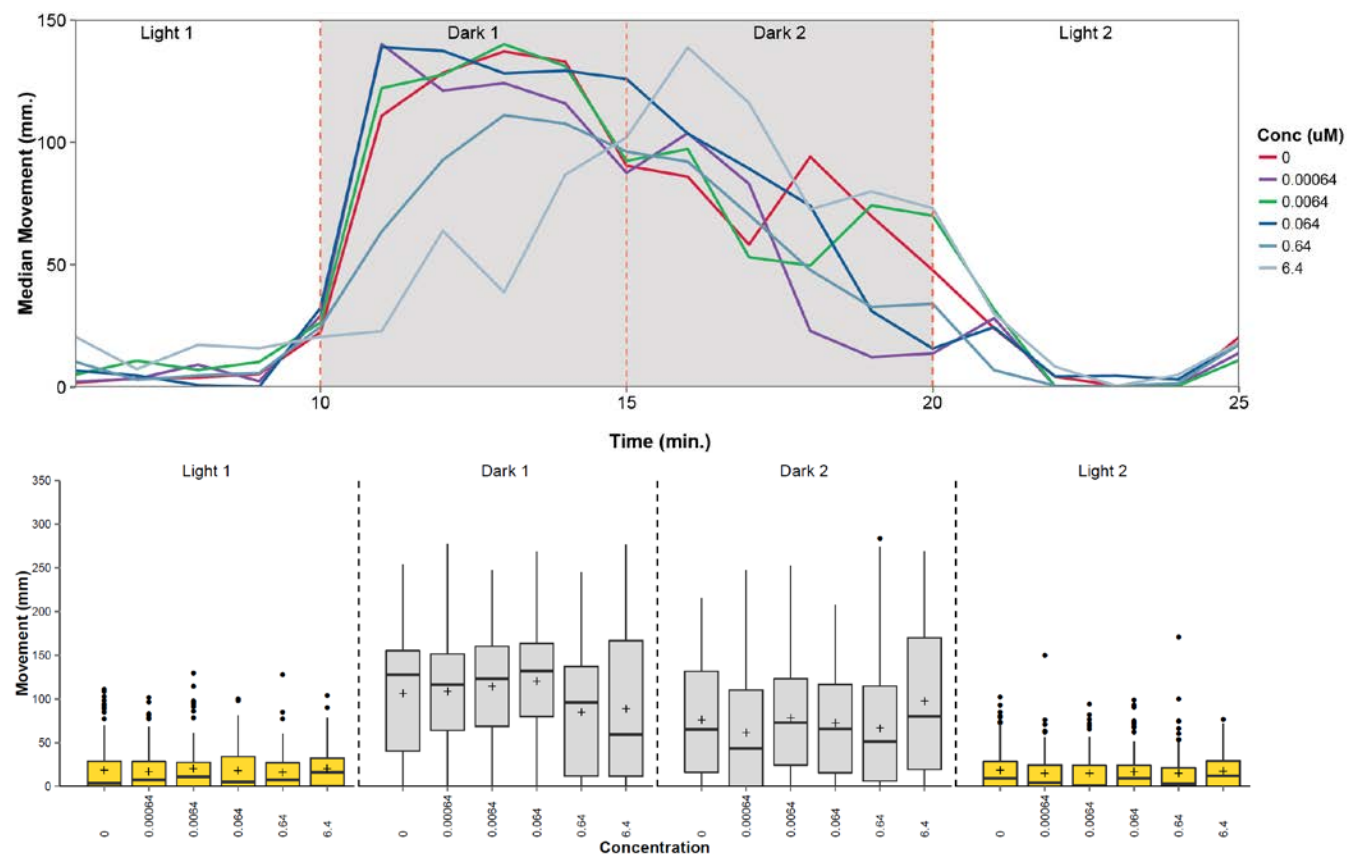
2,4,6-TBP: Zebrafish Mortality and Malformations at 24 and 120 hpf



2,4,6-TBP: Zebrafish embryo PRAT results at 24 hpf

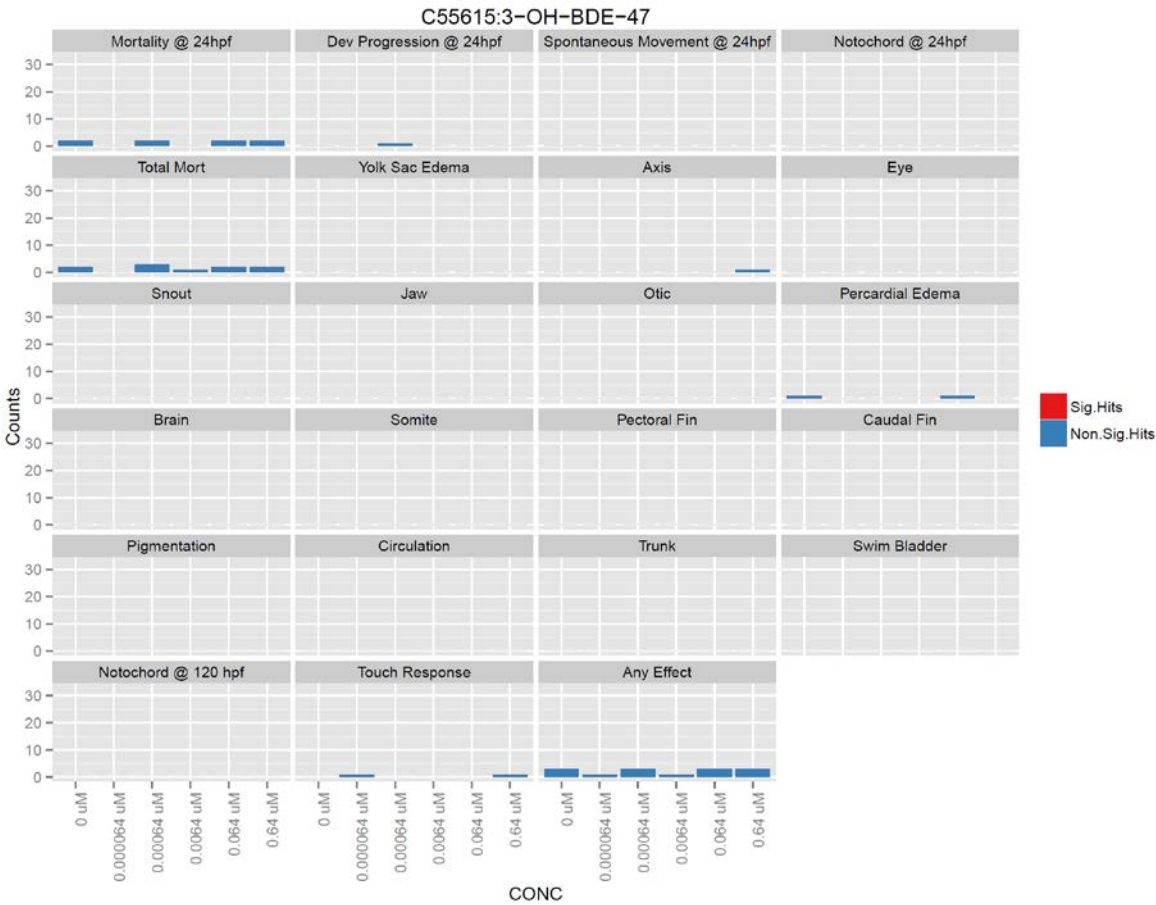


**2,4,6-TBP: Zebrafish larvae PMR assay results at 120 hpf**

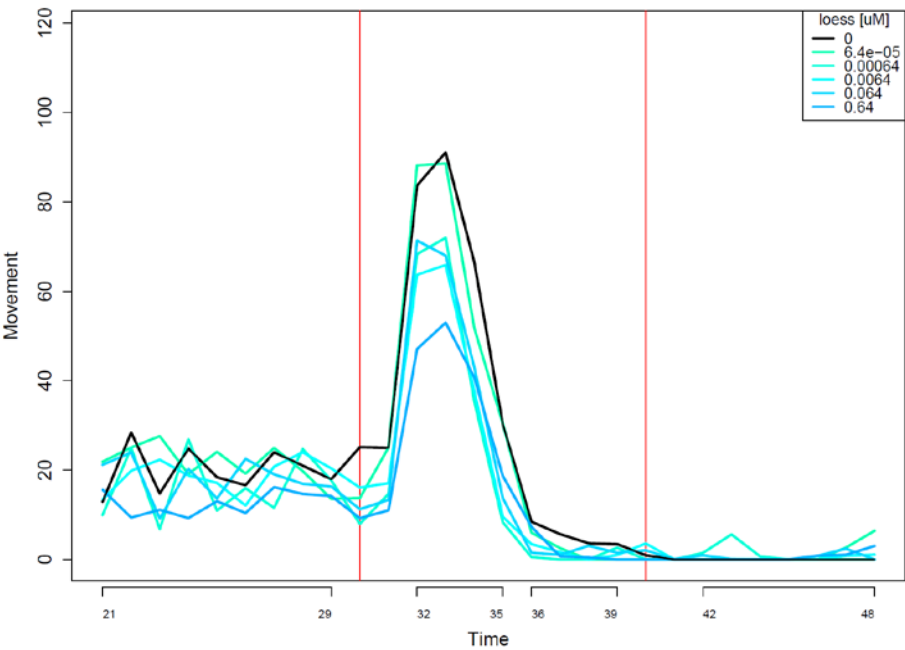


**3-Hydroxy-2,2',4,4'-tetraBDE (3-OH-BDE-47)**

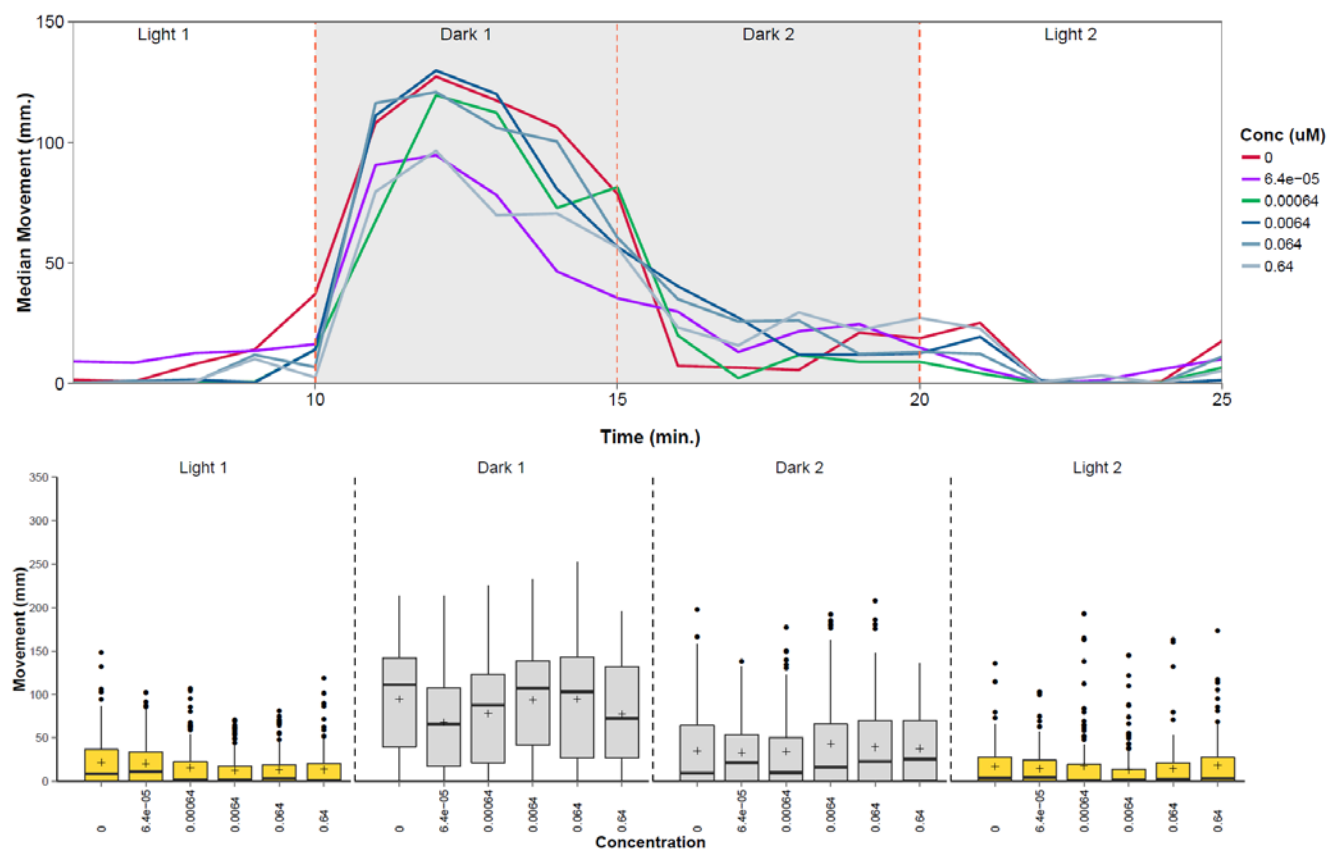
**3-OH-BDE-47: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**3-OH-BDE-47: Zebrafish embryo PRAT results at 24 hpf**

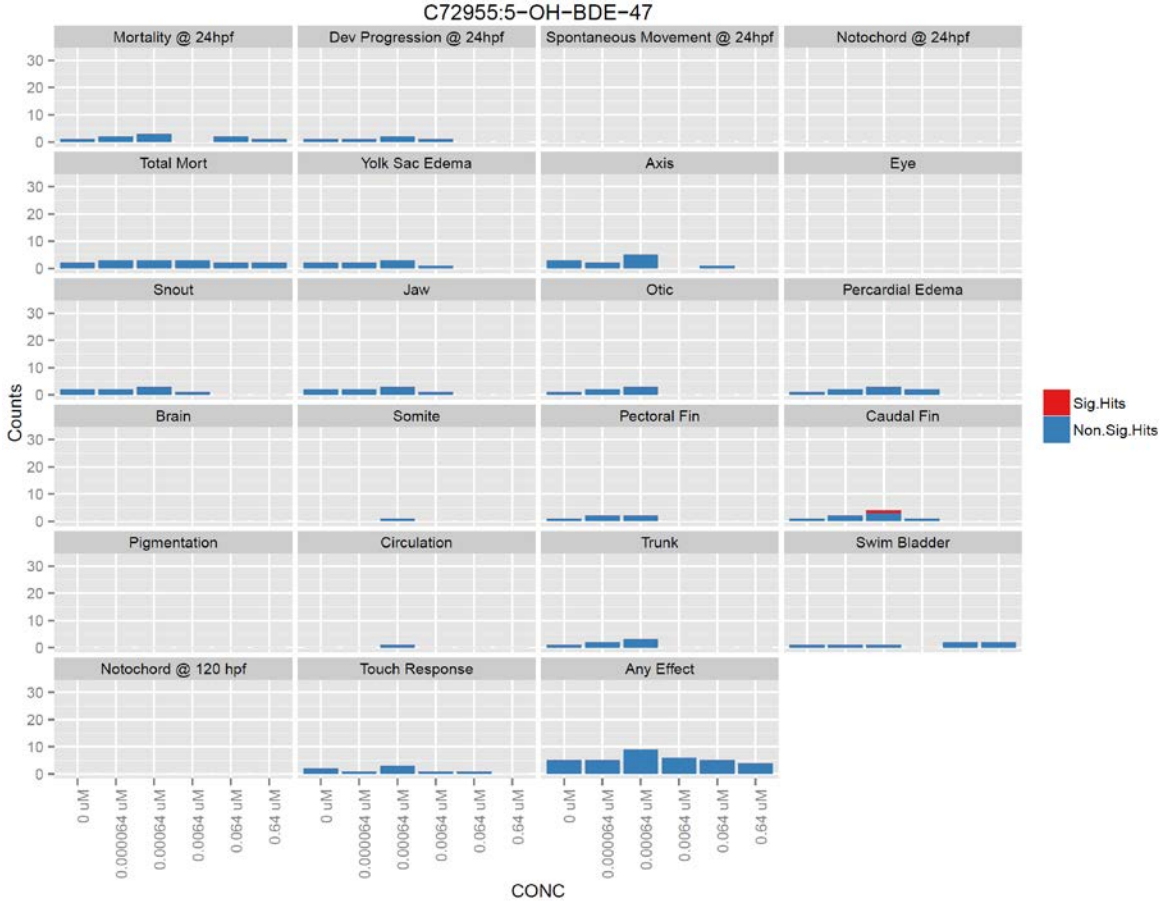


**3-OH-BDE-47: Zebrafish larvae PMR assay results at 120 hpf**

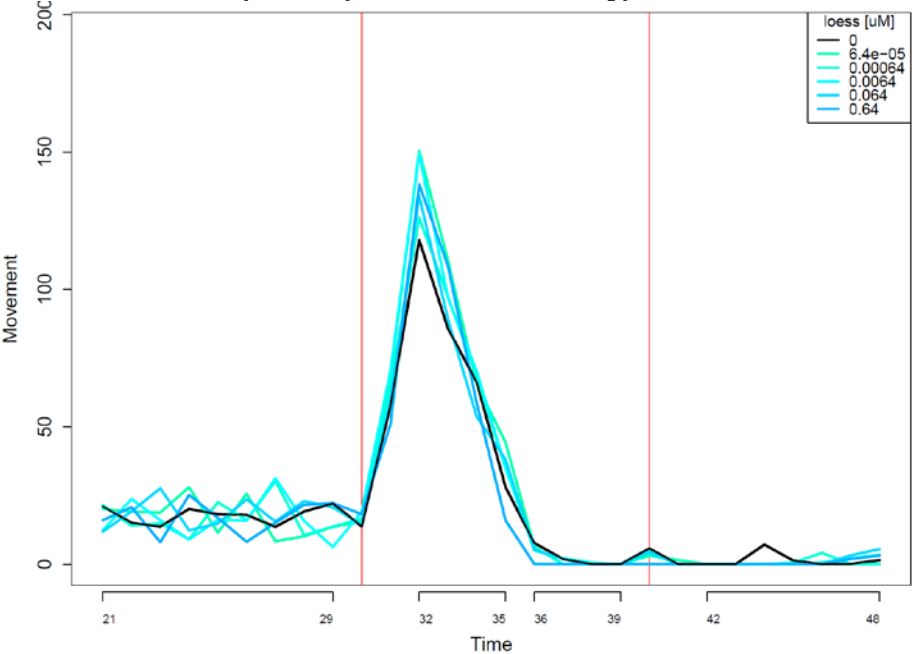


**5-Hydroxy-2,2',4,4'-tetraBDE (5-OH-BDE-47)**

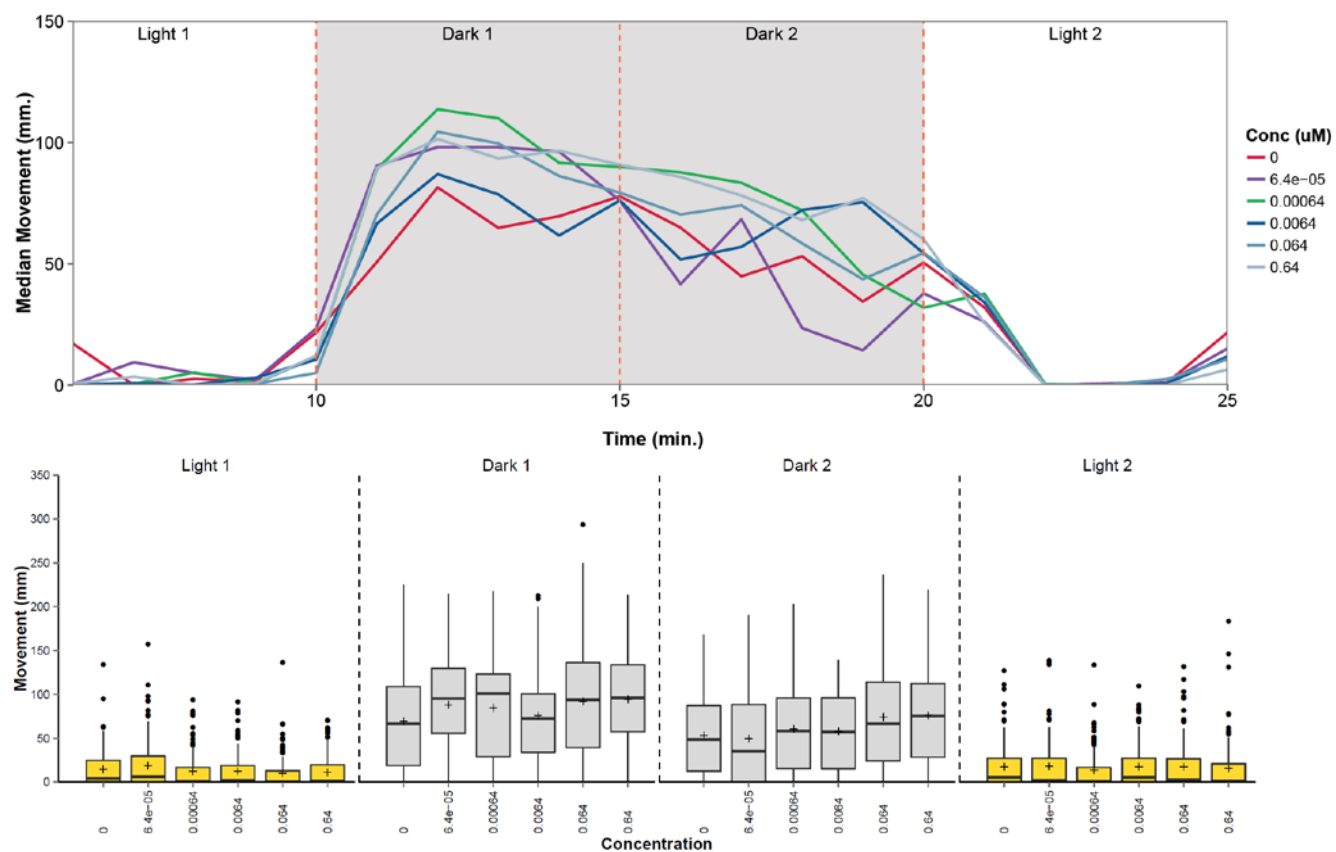
**5-OH-BDE-47: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**5-OH-BDE-47: Zebrafish embryo PRAT results at 24 hpf**



**5-OH-BDE-47: Zebrafish larvae PMR assay results at 120 hpf**

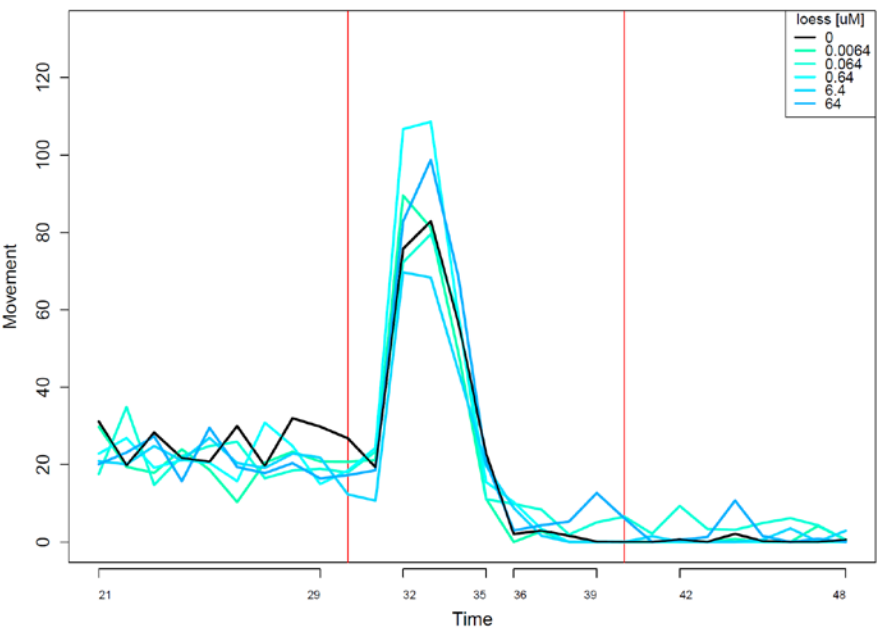


**6-Hydroxy-2,2',4,4'-tetraBDE (6-OH-BDE-47)**

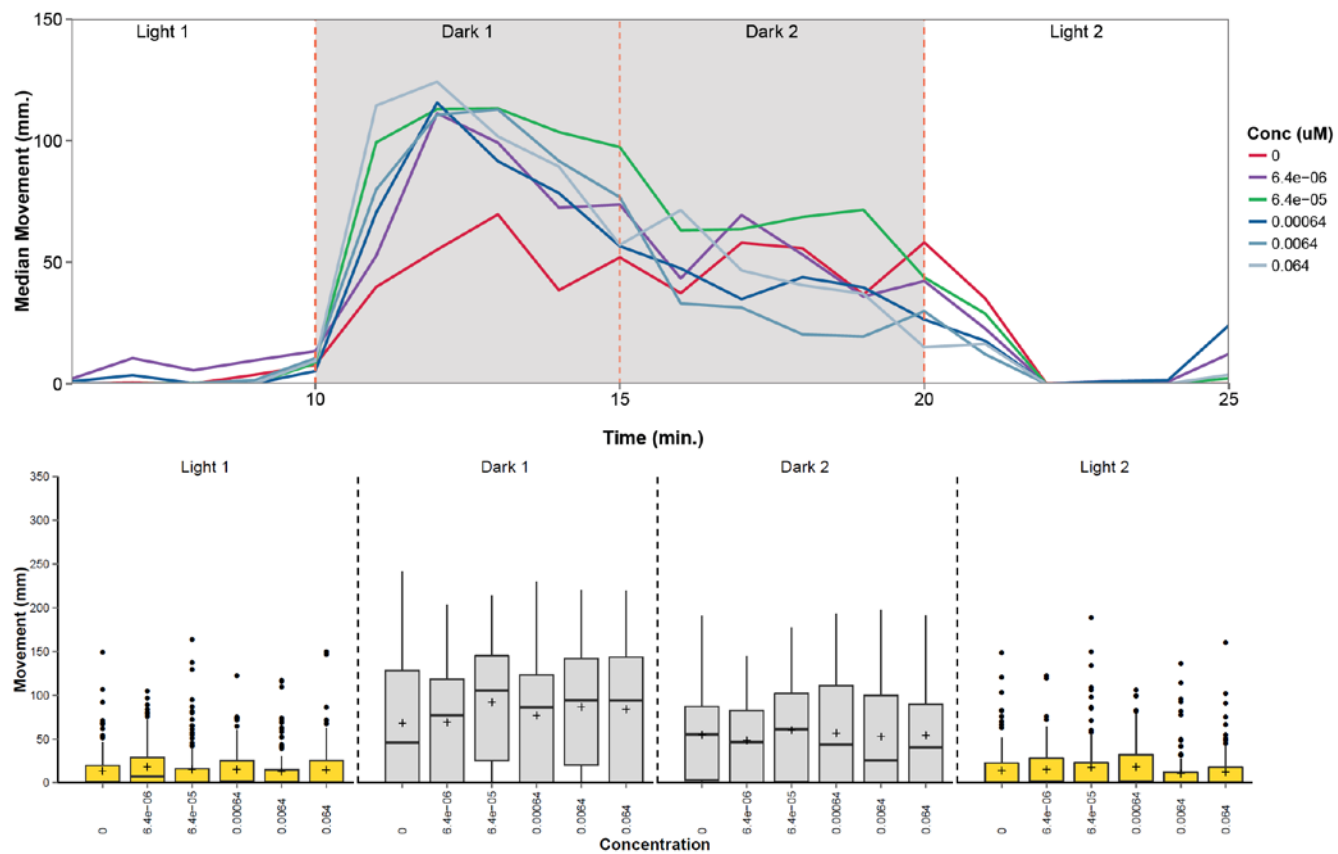
**6-OH-BDE-47: Zebrafish Mortality and Malformations at 24 and 120 hpf**



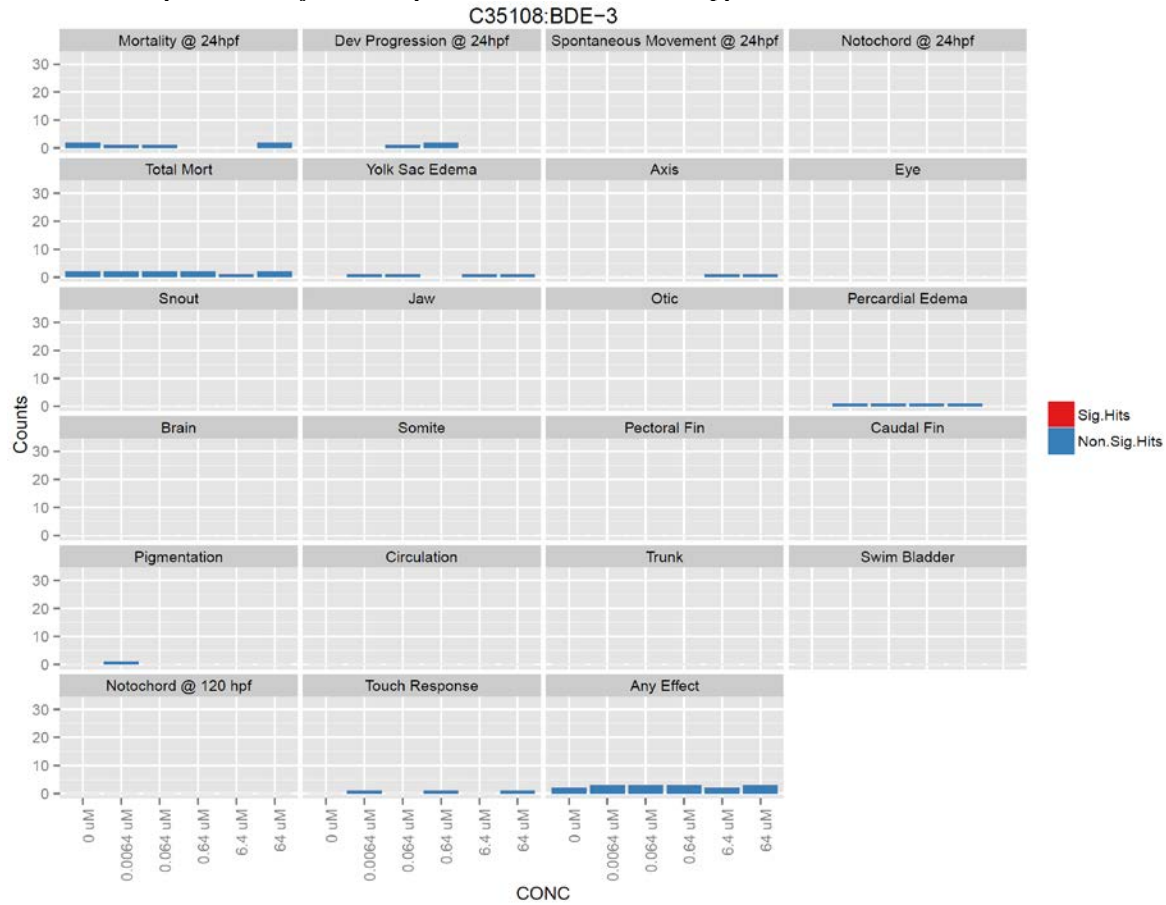
**6-OH-BDE-47: Zebrafish embryo PRAT results at 24 hpf**



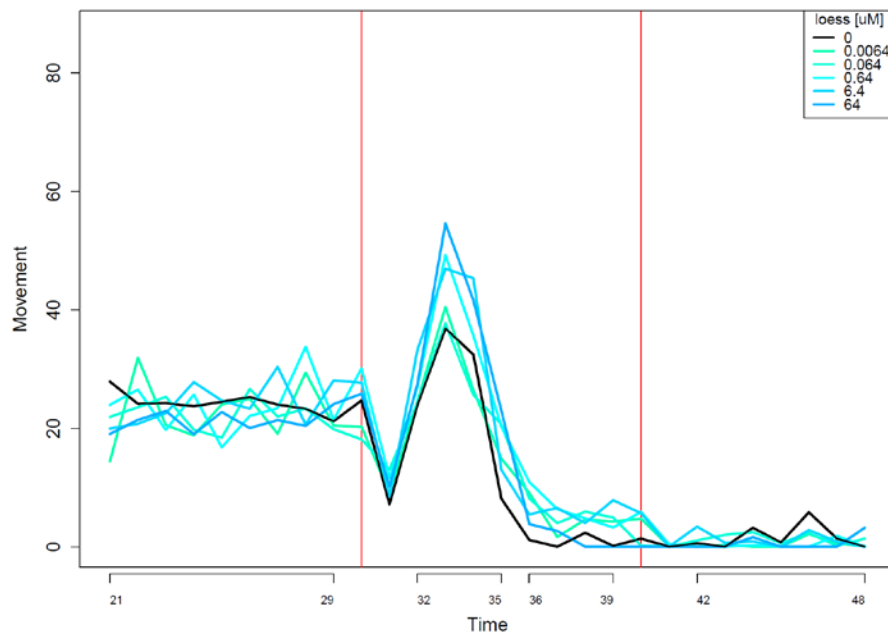
**6-OH-BDE-47: Zebrafish larvae PMR assay results at 120 hpf**



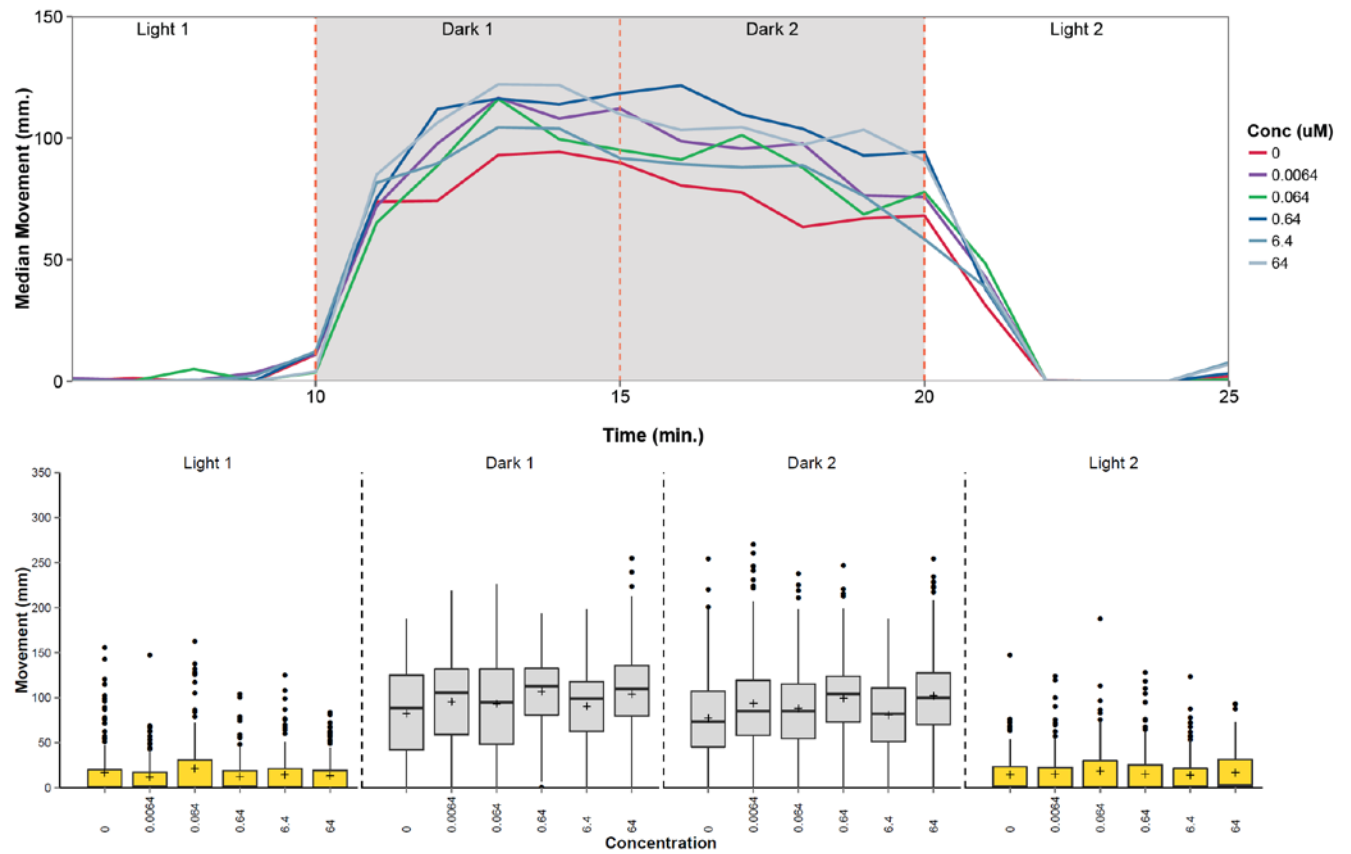
### ***BDE-3: Zebrafish Mortality and Malformations at 24 and 120 hpf***



### ***BDE-3: Zebrafish embryo PRAT results at 24 hpf***



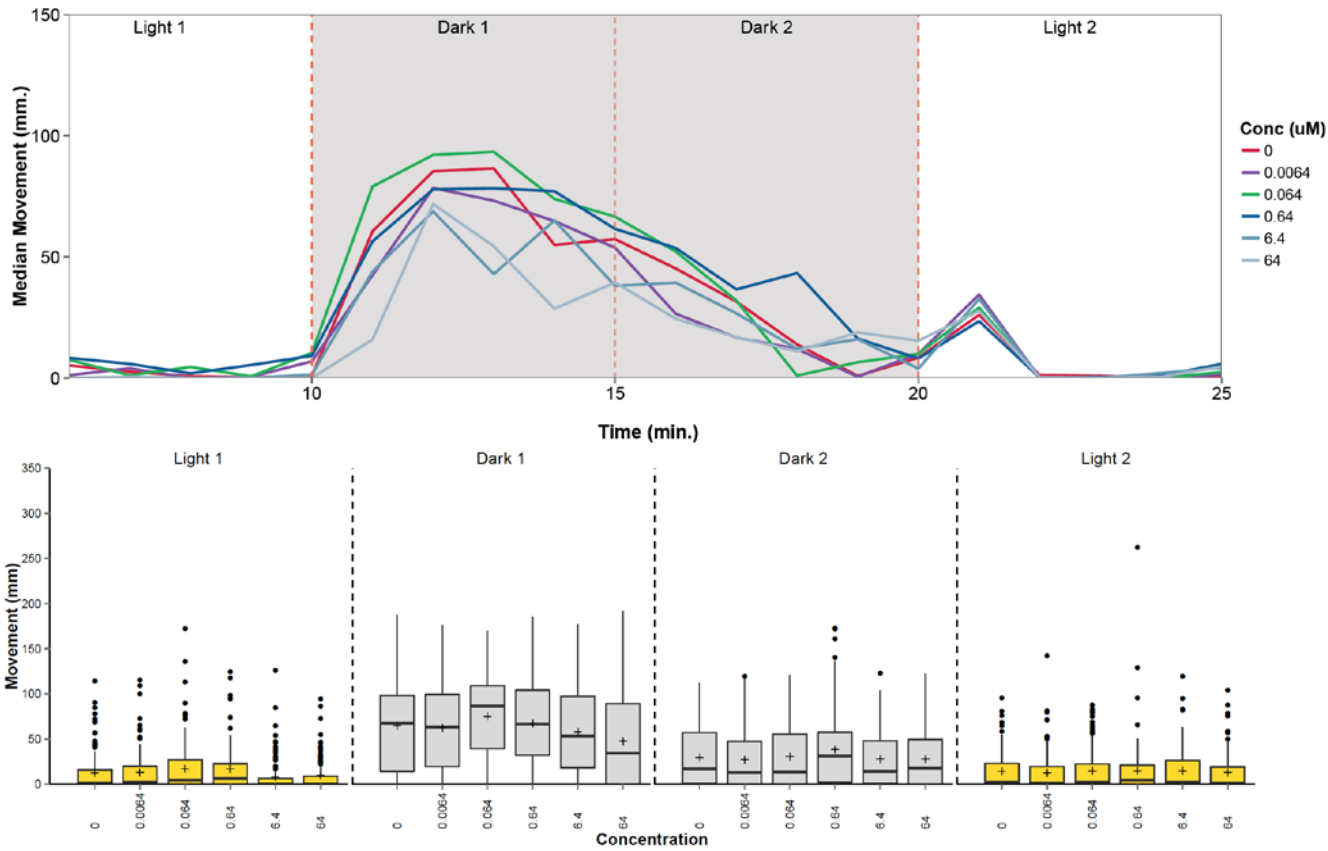
**BDE-3: Zebrafish larvae PMR assay results at 120 hpf**



***BDE-15: Zebrafish Mortality and Malformations at 24 and 120 hpf***



*BDE-15: Zebrafish larvae PMR assay results at 120 hpf*



***BDE-47: Zebrafish Mortality and Malformations at 24 and 120 hpf***

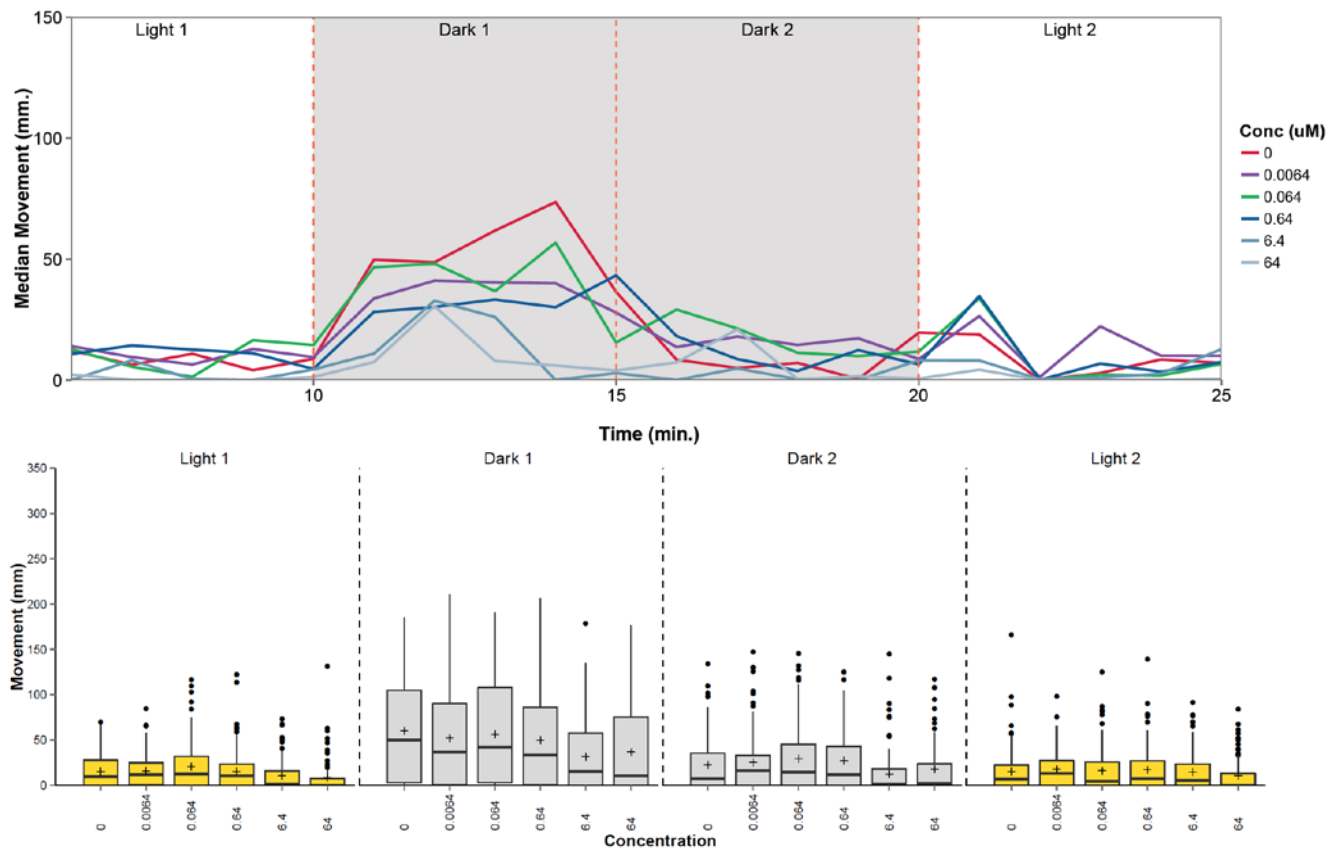
Figure 1 displays a grid of 24 bar charts showing the number of significant (red) and non-significant (blue) hits for various phenotypes across different concentrations of a chemical. The y-axis represents 'Counts' (0 to 30), and the x-axis represents 'CONC' (0 uM, 0.0064 uM, 0.064 uM, 0.64 uM, 6.4 uM, 64 uM). The phenotypes are arranged in a 6x4 grid. A legend indicates that red bars represent 'Sig. Hits' and blue bars represent 'Non.Sig. Hits'.

| Phenotype                    | 0 uM | 0.0064 uM | 0.064 uM | 0.64 uM | 6.4 uM | 64 uM |
|------------------------------|------|-----------|----------|---------|--------|-------|
| Mortality @ 24hpf            | 0    | 0         | 5        | 3       | 3      | 3     |
| Dev Progression @ 24hpf      | 0    | 0         | 0        | 0       | 0      | 0     |
| Spontaneous Movement @ 24hpf | 0    | 0         | 0        | 0       | 0      | 0     |
| Notochord @ 24hpf            | 0    | 0         | 0        | 0       | 0      | 0     |
| Total Mort                   | 2    | 7         | 6        | 6       | 5      | 4     |
| Yolk Sac Edema               | 0    | 0         | 0        | 0       | 0      | 0     |
| Axis                         | 0    | 0         | 0        | 0       | 0      | 0     |
| Eye                          | 0    | 0         | 0        | 0       | 0      | 0     |
| Snout                        | 0    | 0         | 0        | 0       | 0      | 0     |
| Jaw                          | 0    | 0         | 0        | 0       | 0      | 0     |
| Otic                         | 0    | 0         | 0        | 0       | 0      | 0     |
| Pericardial Edema            | 0    | 0         | 0        | 0       | 0      | 0     |
| Brain                        | 0    | 0         | 0        | 0       | 0      | 0     |
| Somite                       | 0    | 0         | 0        | 0       | 0      | 0     |
| Pectoral Fin                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Caudal Fin                   | 0    | 0         | 0        | 0       | 0      | 0     |
| Pigmentation                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Circulation                  | 0    | 0         | 0        | 0       | 0      | 0     |
| Trunk                        | 0    | 0         | 0        | 0       | 0      | 0     |
| Swim Bladder                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Notochord @ 120 hpf          | 0    | 0         | 0        | 0       | 0      | 0     |
| Touch Response               | 0    | 0         | 0        | 0       | 0      | 0     |
| Any Effect                   | 0    | 0         | 0        | 0       | 0      | 0     |

The graph displays movement data over time for five different concentrations of loess. The y-axis represents 'Movement' from 0 to 100, and the x-axis represents 'Time' from 21 to 48. A black line represents the 0  $\mu\text{M}$  concentration, while blue lines of increasing intensity represent concentrations of 0.0064, 0.064, 0.64, and 6.4  $\mu\text{M}$ . All concentrations show a similar trend with a sharp increase in movement starting around time 30, peaking between times 32 and 34, and then declining. The peak movement values increase with the concentration of loess. Two vertical red lines are positioned at time 31 and time 40.

| Time | 0 $\mu\text{M}$ | 0.0064 $\mu\text{M}$ | 0.064 $\mu\text{M}$ | 0.64 $\mu\text{M}$ | 6.4 $\mu\text{M}$ |
|------|-----------------|----------------------|---------------------|--------------------|-------------------|
| 21   | 26              | 18                   | 20                  | 22                 | 35                |
| 25   | 25              | 20                   | 22                  | 24                 | 26                |
| 29   | 24              | 18                   | 20                  | 22                 | 33                |
| 31   | 10              | 10                   | 10                  | 10                 | 10                |
| 32   | 60              | 50                   | 55                  | 60                 | 68                |
| 33   | 60              | 55                   | 60                  | 65                 | 68                |
| 34   | 45              | 40                   | 45                  | 50                 | 55                |
| 35   | 25              | 20                   | 25                  | 30                 | 35                |
| 36   | 10              | 5                    | 10                  | 15                 | 20                |
| 37   | 5               | 2                    | 5                   | 10                 | 15                |
| 38   | 5               | 2                    | 5                   | 10                 | 15                |
| 39   | 5               | 2                    | 5                   | 10                 | 15                |
| 40   | 2               | 1                    | 2                   | 5                  | 10                |
| 41   | 1               | 1                    | 2                   | 5                  | 10                |
| 42   | 1               | 1                    | 2                   | 5                  | 10                |
| 43   | 1               | 1                    | 2                   | 5                  | 10                |
| 44   | 1               | 1                    | 2                   | 5                  | 10                |
| 45   | 1               | 1                    | 2                   | 5                  | 10                |
| 46   | 1               | 1                    | 2                   | 5                  | 10                |
| 47   | 1               | 1                    | 2                   | 5                  | 10                |
| 48   | 1               | 1                    | 2                   | 5                  | 10                |

**BDE-47: Zebrafish larvae PMR assay results at 120 hpf**



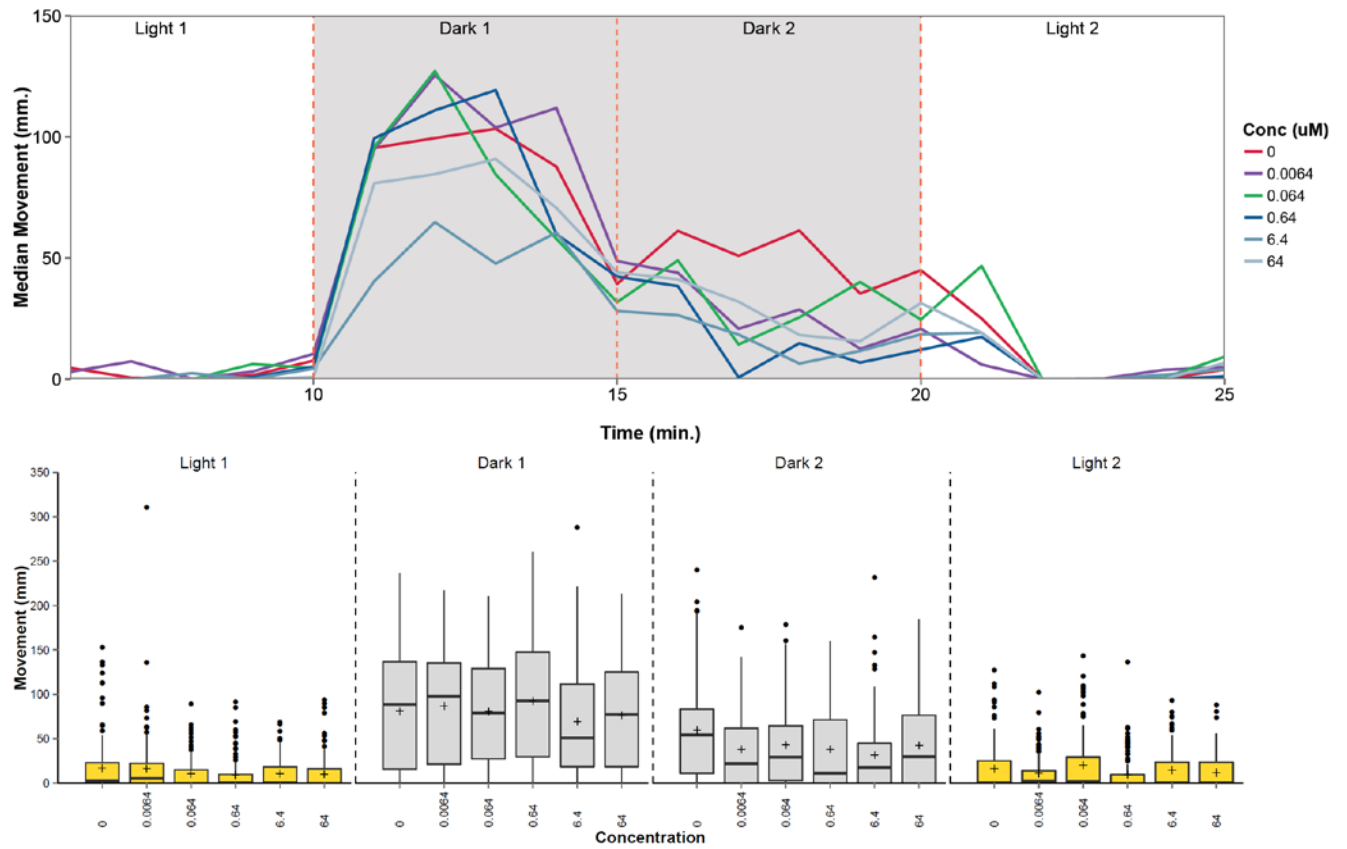
***BDE-99: Zebrafish Mortality and Malformations at 24 and 120 hpf***

Figure 1 displays a grid of 28 bar charts showing the number of significant (red) and non-significant (blue) hits for various phenotypes across different concentrations of a treatment. The y-axis represents 'Counts' (0 to 30), and the x-axis represents 'CONC' (0 uM, 0.0064 uM, 0.064 uM, 0.64 uM, 6.4 uM, 64 uM). The phenotypes are organized into four columns and seven rows. A legend indicates that red bars represent 'Sig. Hits' and blue bars represent 'Non.Sig. Hits'.

| Phenotype                    | 0 uM | 0.0064 uM | 0.064 uM | 0.64 uM | 6.4 uM | 64 uM |
|------------------------------|------|-----------|----------|---------|--------|-------|
| Mortality @ 24hpf            | 0    | 0         | 0        | 0       | 3      | 0     |
| Dev Progression @ 24hpf      | 0    | 0         | 0        | 0       | 0      | 2     |
| Spontaneous Movement @ 24hpf | 0    | 0         | 0        | 0       | 0      | 0     |
| Notochord @ 24hpf            | 0    | 0         | 0        | 0       | 0      | 0     |
| Total Mort                   | 2    | 2         | 7        | 3       | 4      | 2     |
| Yolk Sac Edema               | 0    | 0         | 0        | 0       | 0      | 0     |
| Axis                         | 0    | 0         | 0        | 0       | 0      | 0     |
| Eye                          | 0    | 0         | 0        | 0       | 0      | 0     |
| Snout                        | 0    | 1         | 0        | 0       | 0      | 1     |
| Jaw                          | 0    | 0         | 0        | 0       | 0      | 0     |
| Otic                         | 0    | 0         | 0        | 0       | 0      | 0     |
| Pericardial Edema            | 0    | 0         | 0        | 0       | 2      | 2     |
| Brain                        | 0    | 1         | 0        | 0       | 0      | 0     |
| Somite                       | 0    | 0         | 0        | 0       | 0      | 0     |
| Pectoral Fin                 | 0    | 0         | 0        | 0       | 0      | 1     |
| Caudal Fin                   | 0    | 0         | 0        | 0       | 1      | 0     |
| Pigmentation                 | 0    | 0         | 0        | 0       | 1      | 0     |
| Circulation                  | 0    | 0         | 0        | 0       | 0      | 0     |
| Trunk                        | 0    | 1         | 0        | 0       | 0      | 0     |
| Swim Bladder                 | 0    | 0         | 0        | 0       | 3      | 2     |
| Notochord @ 120 hpf          | 0    | 0         | 0        | 0       | 0      | 0     |
| Touch Response               | 0    | 1         | 0        | 0       | 0      | 0     |
| Any Effect                   | 2    | 2         | 7        | 7       | 7      | 4     |

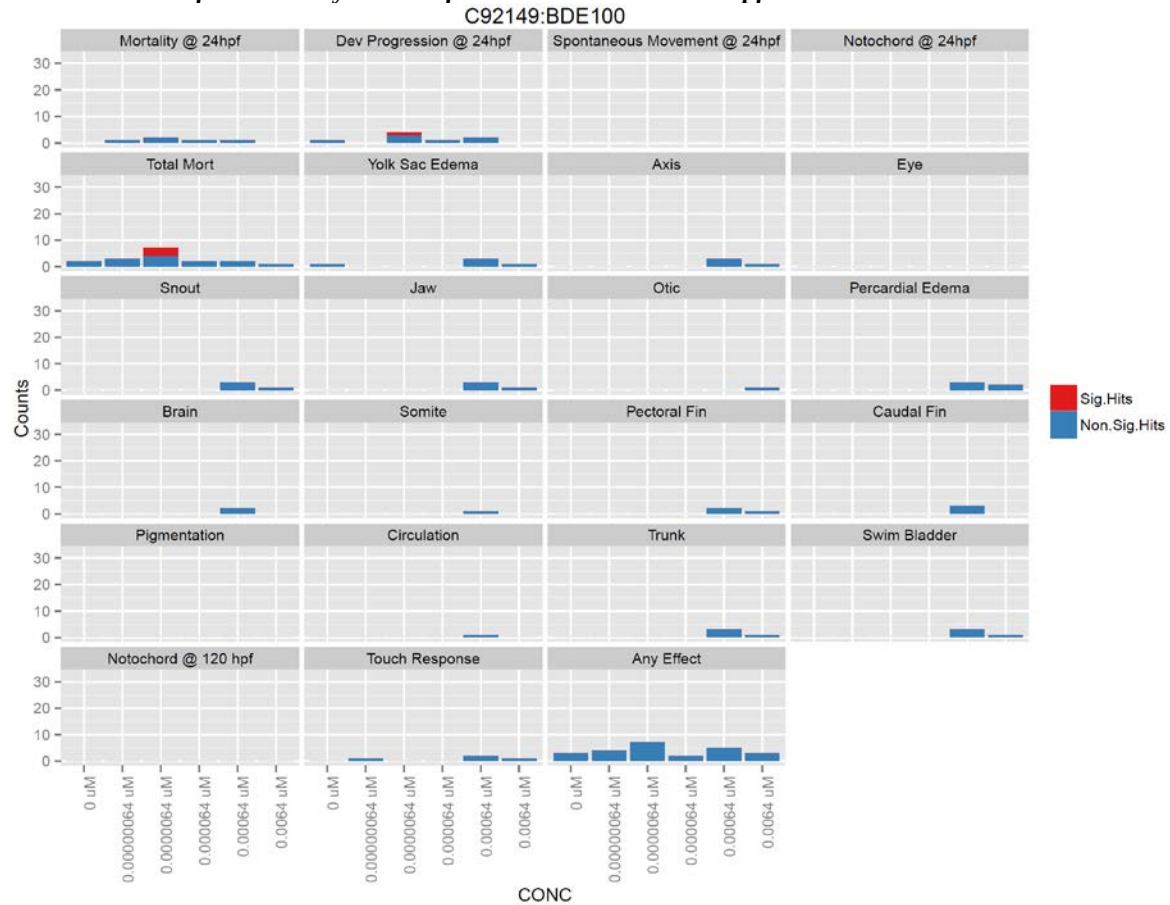
The graph displays movement data over time for various loess concentrations. The x-axis represents time from 21 to 48, and the y-axis represents movement from 0 to 140. A legend in the top right corner identifies the loess concentrations: 0 (black line), 0.0064 (dark green), 0.064 (medium green), 0.64 (light green), 6.4 (cyan), and 64 (blue). All lines show a similar trend with a peak around time 33. The peak movement increases with higher loess concentration, with the 0.064 concentration reaching the highest peak of approximately 95. Two vertical red lines are drawn at time 30 and time 40.

**BDE-99: Zebrafish larvae PMR assay results at 120 hpf**

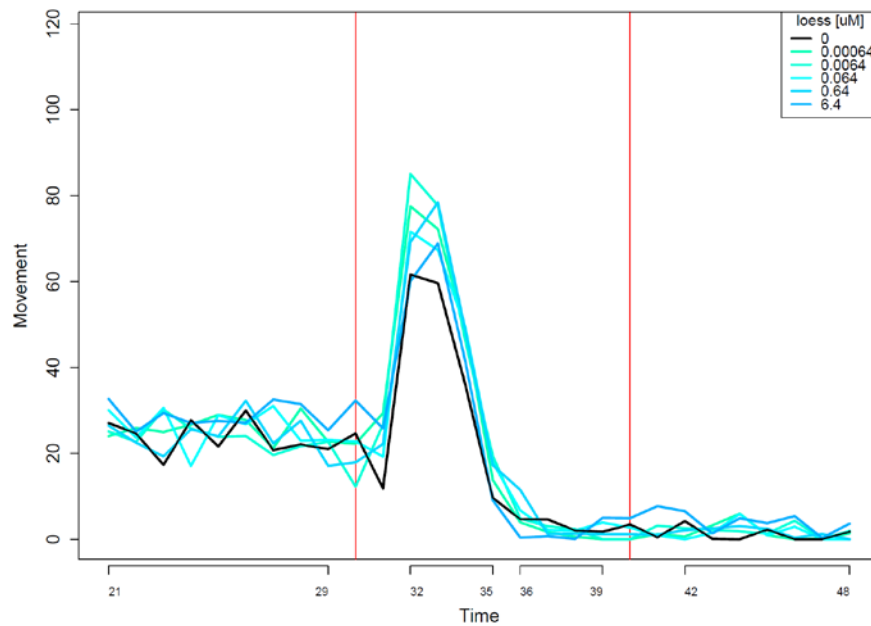


## 2,2',4,4',6-Pentabromodiphenyl ether (BDE-100)

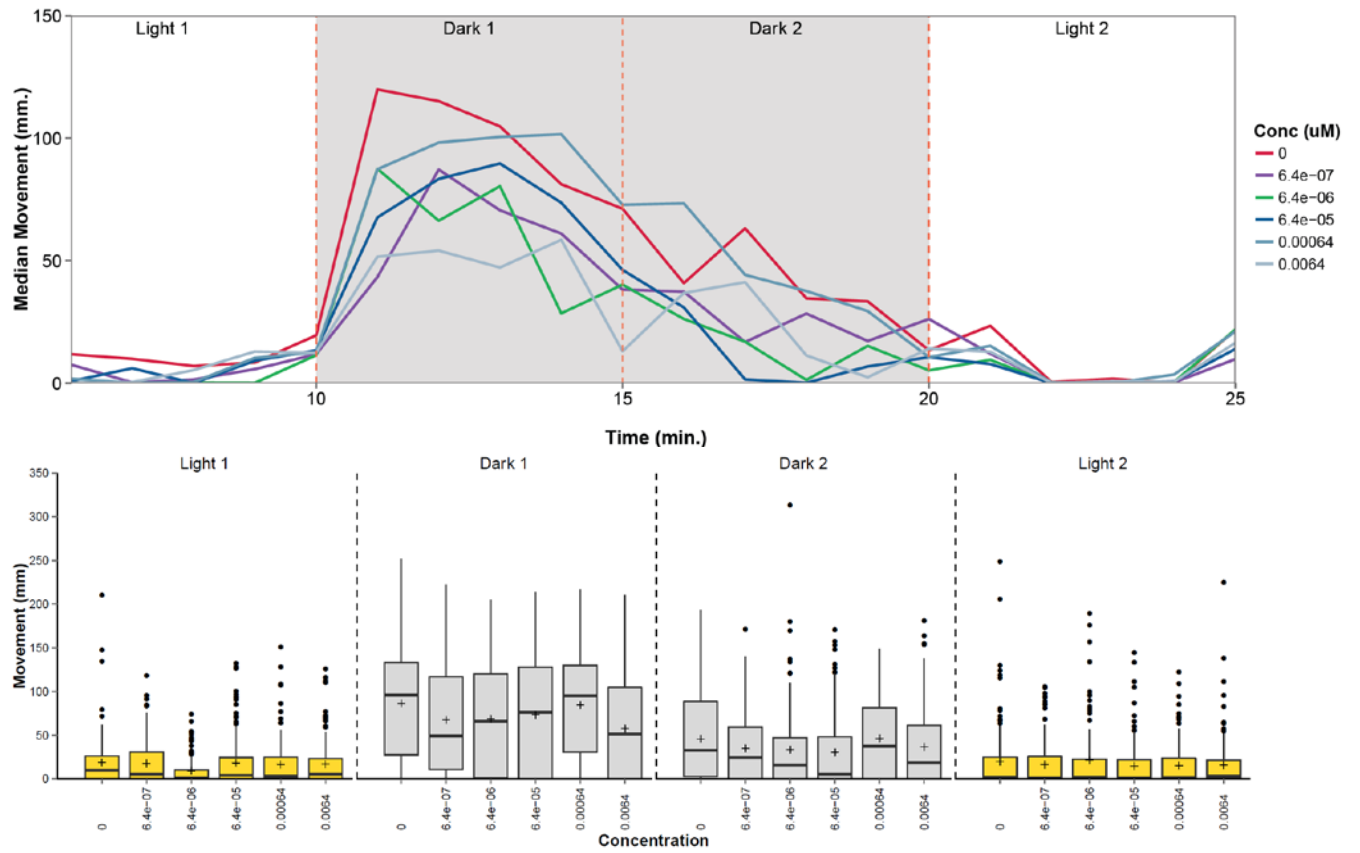
### BDE-100: Zebrafish Mortality and Malformations at 24 and 120 hpf



### BDE-100: Zebrafish embryo PRAT results at 24 hpf



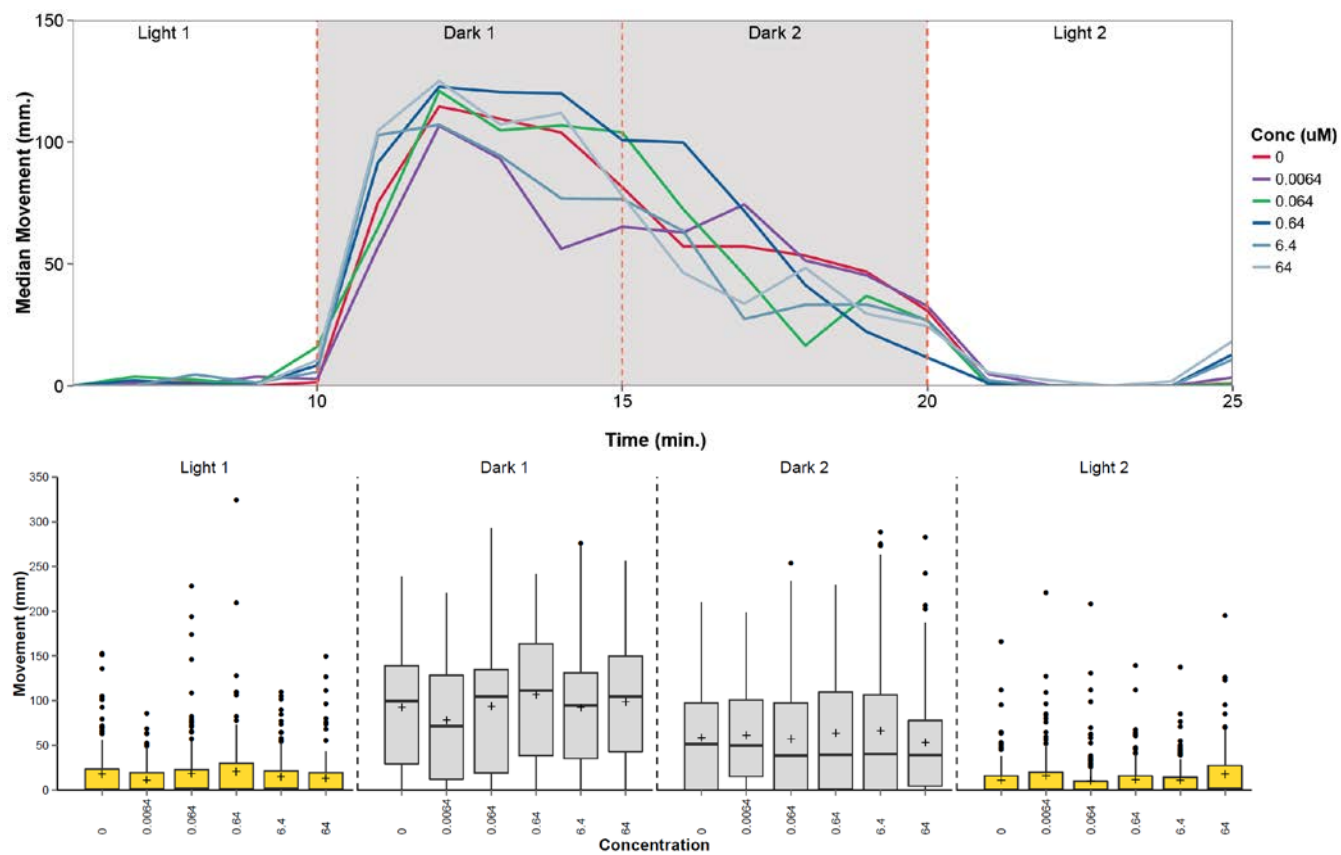
**BDE-100: Zebrafish larvae PMR assay results at 120 hpf**



C57798:BDE-153

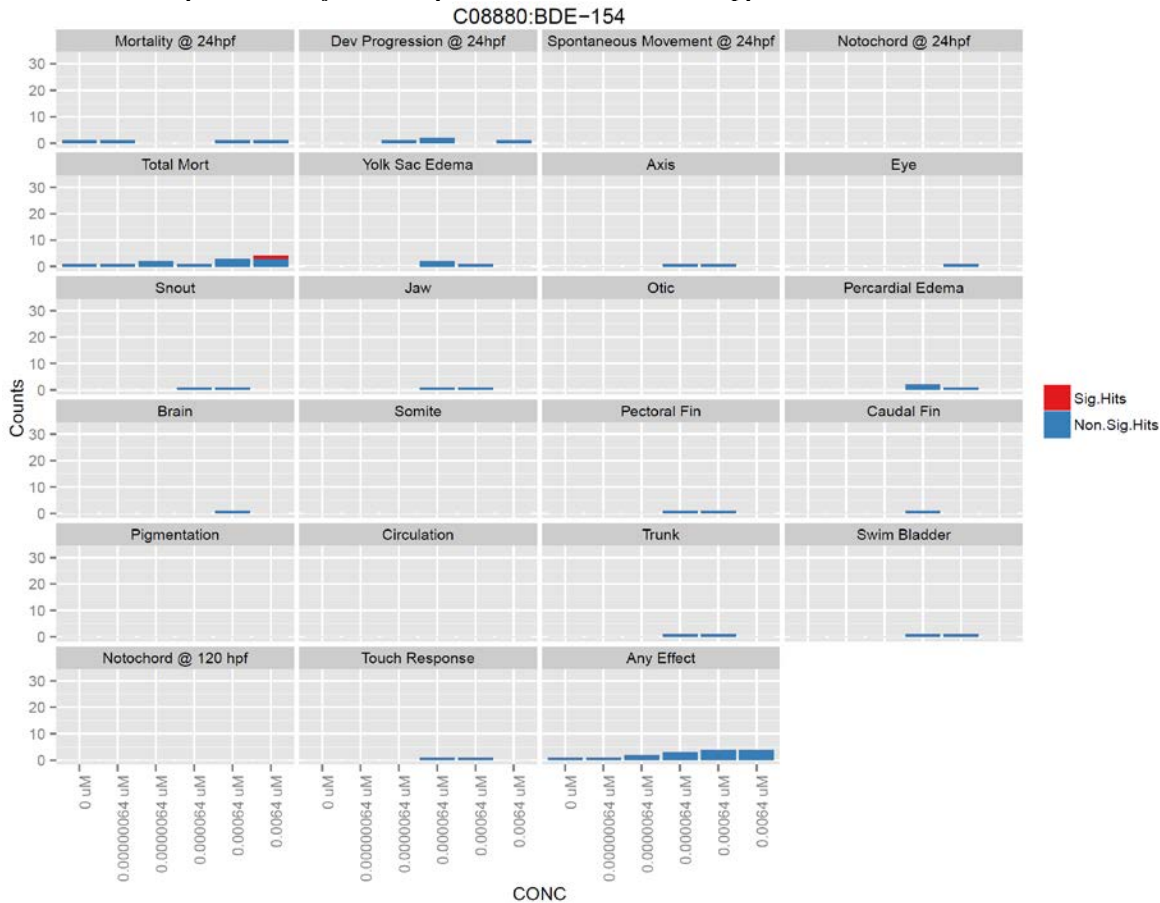


**BDE-153: Zebrafish larvae PMR assay results at 120 hpf**

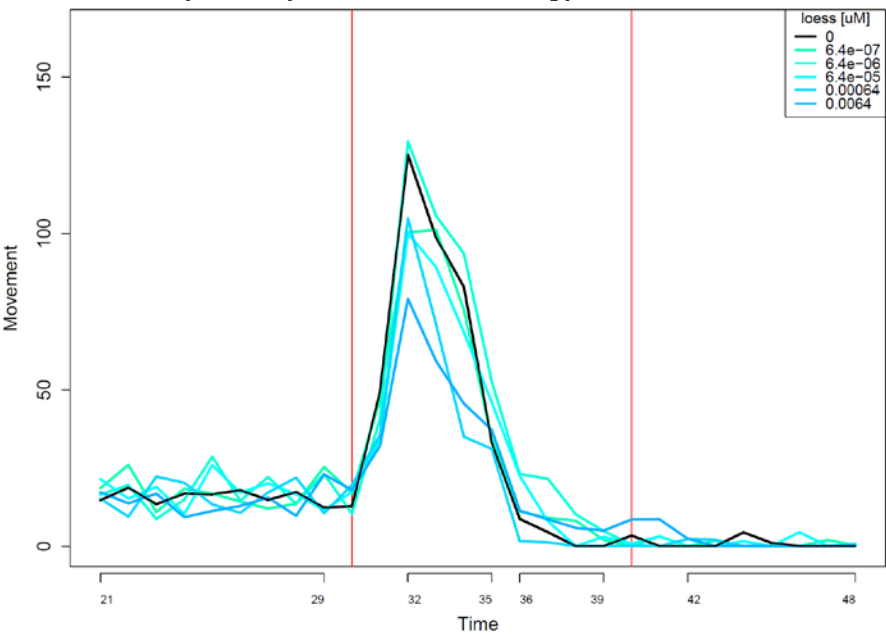


**2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE-154)**

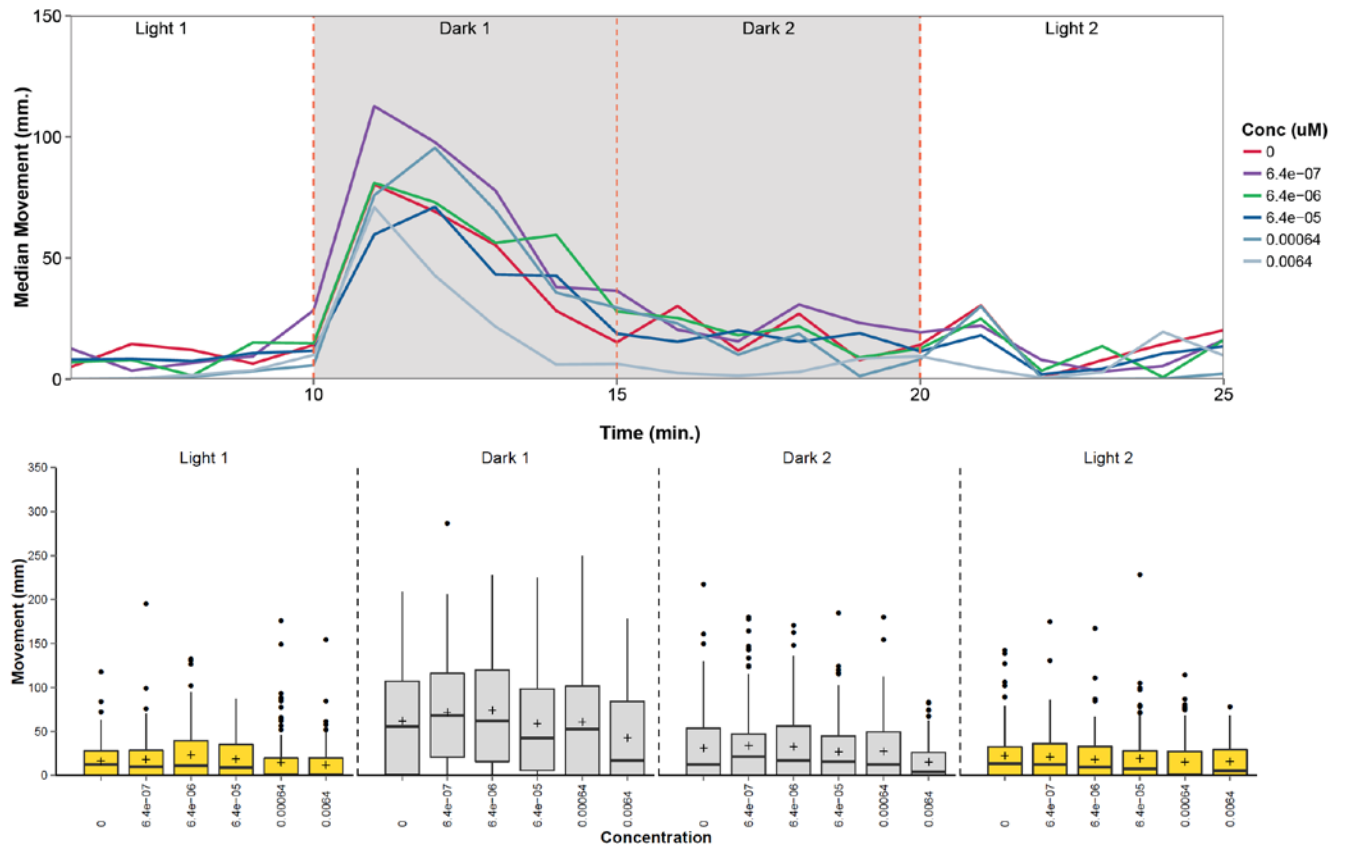
***BDE-154: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***BDE-154: Zebrafish embryo PRAT results at 24 hpf***



**BDE-154: Zebrafish larvae PMR assay results at 120 hpf**

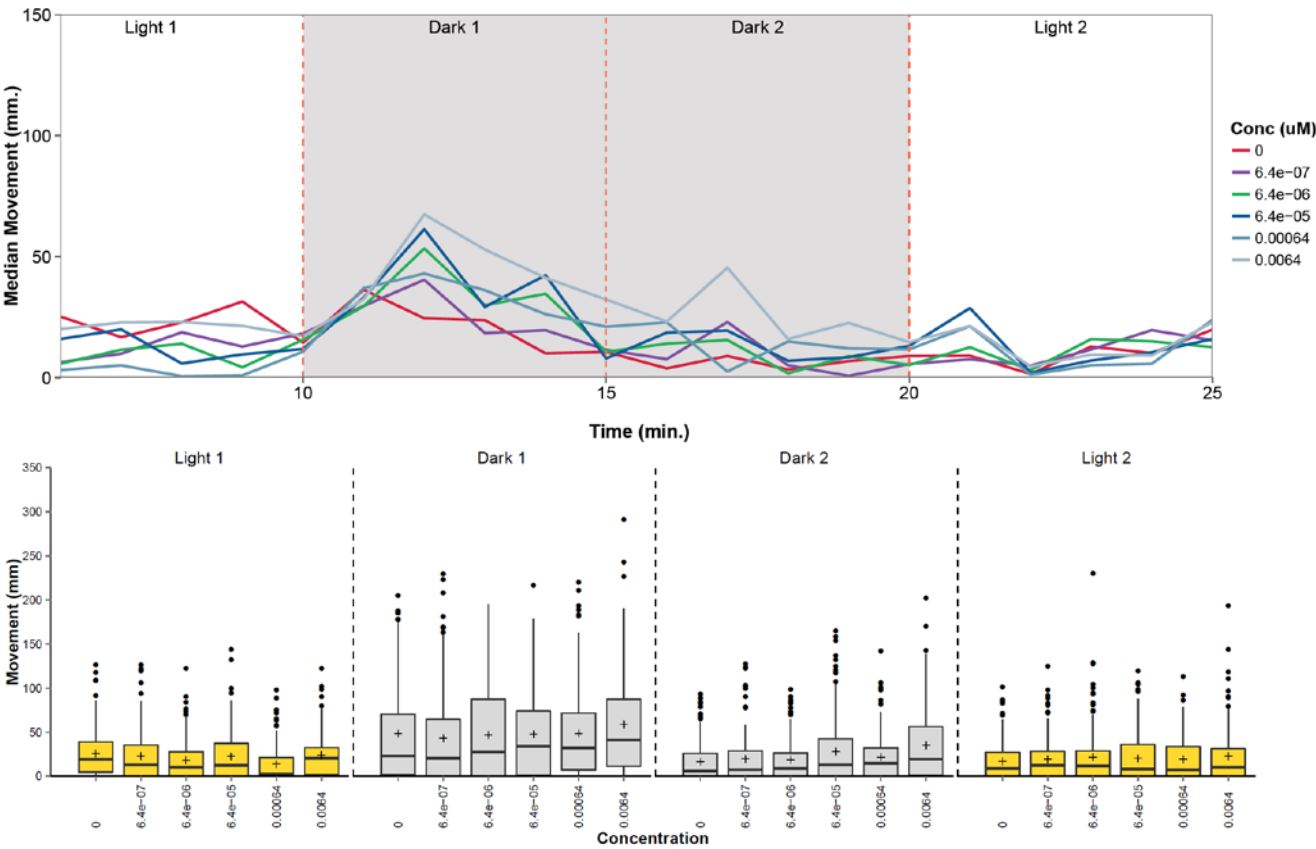


**BDE-183: Zebrafish Mortality and Malformations at 24 and 120 hpf**

C77742:BDE-183



BDE-183: Zebrafish larvae PMR assay results at 120 hpf



***BDE-209: Zebrafish Mortality and Malformations at 24 and 120 hpf***

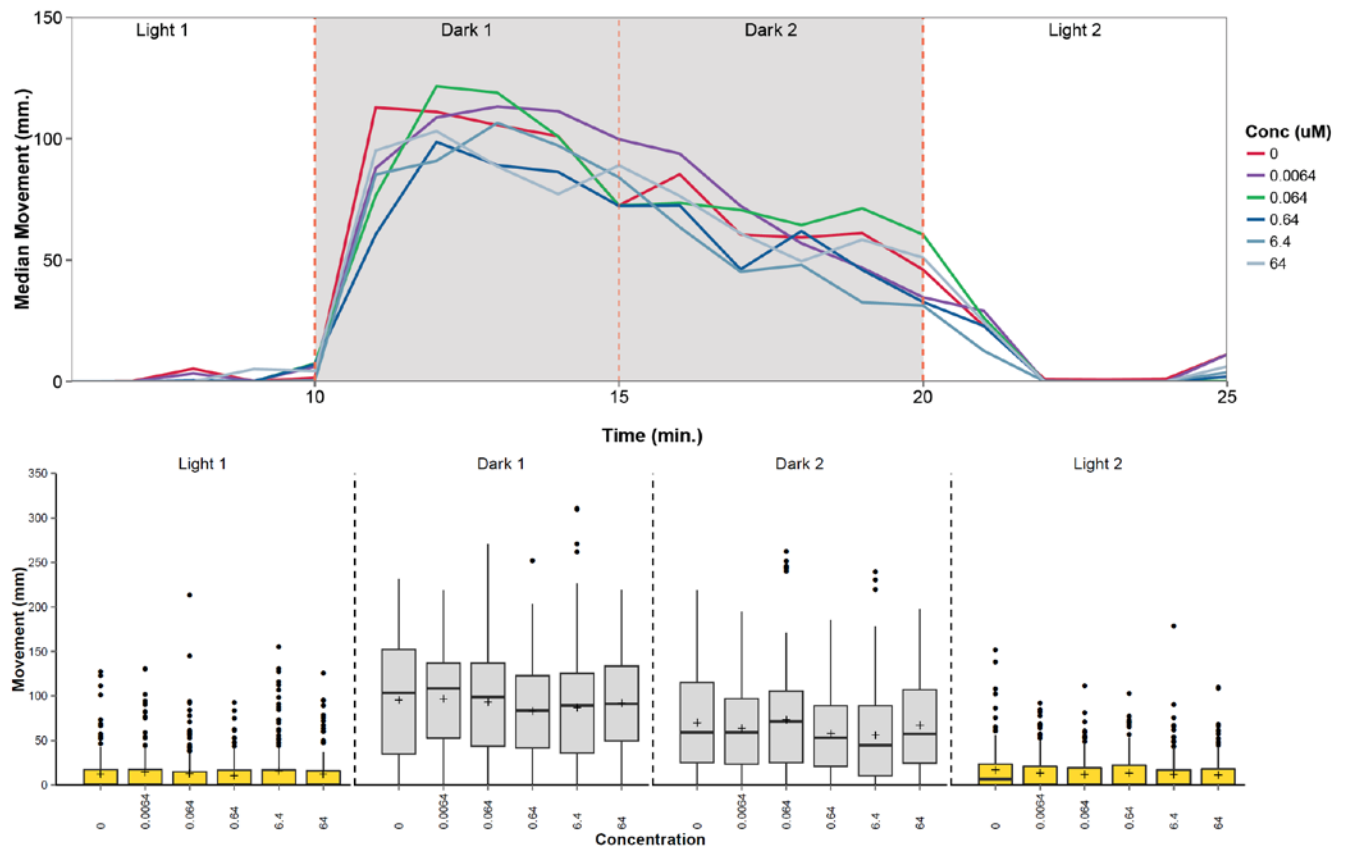
The figure displays a 7x4 grid of dot plots, where each plot represents a specific phenotypic endpoint. The x-axis for all plots is 'CONC' (Concentration) with values: 0 uM, 0.0064 uM, 0.064 uM, 0.64 uM, 6.4 uM, and 64 uM. The y-axis is 'Counts' ranging from 0 to 30. A legend on the right indicates that red bars represent 'Sig.Hits' (Significant Hits) and blue bars represent 'Non.Sig.Hits' (Non-Significant Hits).

The endpoints and their corresponding hit counts are as follows:

| Endpoint                     | 0 uM | 0.0064 uM | 0.064 uM | 0.64 uM | 6.4 uM | 64 uM |
|------------------------------|------|-----------|----------|---------|--------|-------|
| Mortality @ 24hpf            | 0    | 0         | 0        | 0       | 0      | 0     |
| Dev Progression @ 24hpf      | 0    | 0         | 0        | 2       | 0      | 1     |
| Spontaneous Movement @ 24hpf | 0    | 0         | 0        | 0       | 0      | 1     |
| Notochord @ 24hpf            | 0    | 0         | 0        | 0       | 0      | 0     |
| Total Mort                   | 0    | 0         | 0        | 0       | 0      | 1     |
| Yolk Sac Edema               | 0    | 0         | 0        | 2       | 0      | 0     |
| Axis                         | 0    | 0         | 0        | 0       | 2      | 0     |
| Eye                          | 0    | 0         | 0        | 0       | 0      | 0     |
| Snout                        | 0    | 0         | 0        | 2       | 0      | 0     |
| Jaw                          | 0    | 0         | 0        | 2       | 0      | 0     |
| Otic                         | 0    | 0         | 0        | 0       | 0      | 0     |
| Pericardial Edema            | 0    | 0         | 0        | 0       | 1      | 0     |
| Brain                        | 0    | 0         | 0        | 2       | 0      | 0     |
| Somite                       | 0    | 0         | 0        | 2       | 0      | 0     |
| Pectoral Fin                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Caudal Fin                   | 0    | 0         | 0        | 0       | 0      | 0     |
| Pigmentation                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Circulation                  | 0    | 0         | 0        | 0       | 0      | 0     |
| Trunk                        | 0    | 0         | 0        | 0       | 2      | 0     |
| Swim Bladder                 | 0    | 0         | 0        | 0       | 0      | 0     |
| Notochord @ 120 hpf          | 0    | 0         | 0        | 0       | 0      | 0     |
| Touch Response               | 0    | 0         | 0        | 0       | 0      | 0     |
| Any Effect                   | 0    | 0         | 0        | 0       | 0      | 0     |

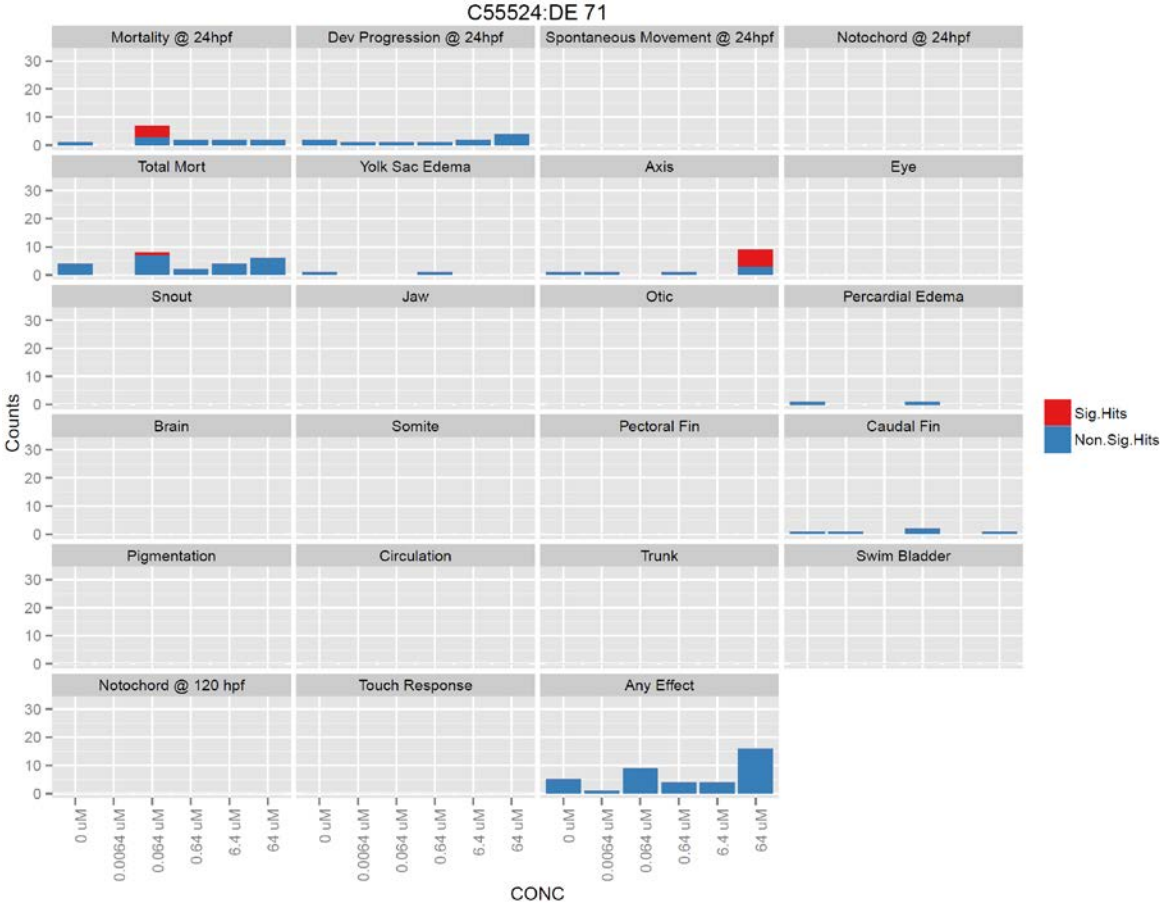
The graph displays Movement (Y-axis, 0 to 140) against Time (X-axis, 21 to 48). A significant peak in movement occurs around time 33, reaching approximately 90 units. Two vertical red lines are positioned at time 30 and time 40. The legend indicates the loess values used for smoothing: 0 (black), 0.0064 (dark green), 0.064 (medium green), 0.64 (light green), 6.4 (cyan), and 64 (blue). The movement is relatively stable and low (around 20 units) before time 30, then spikes sharply, and returns to low levels after time 40.

**BDE-209: Zebrafish larvae PMR assay results at 120 hpf**

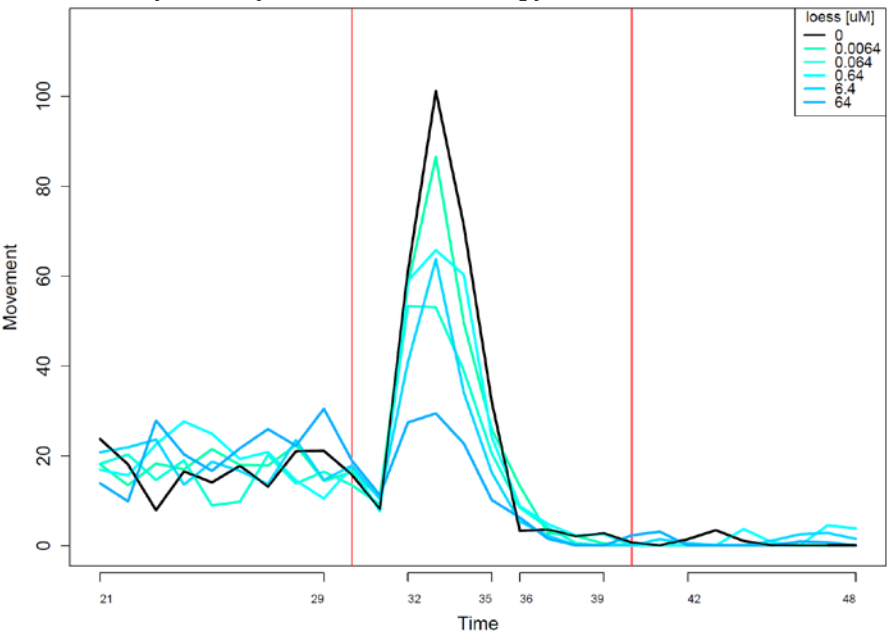


**PentaBDE commercial mixture (DE-71)**

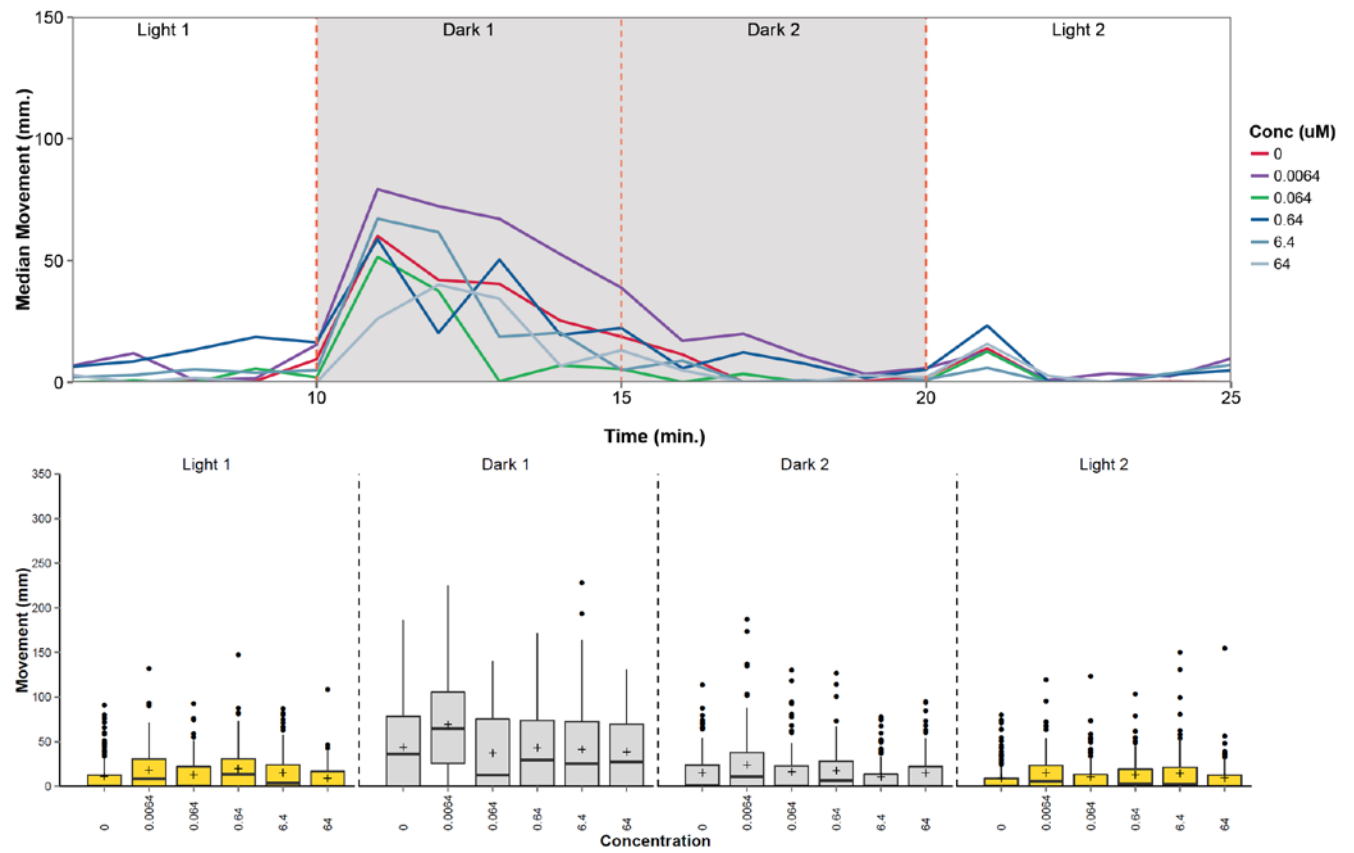
**DE-71: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**DE-71: Zebrafish embryo PRAT results at 24 hpf**



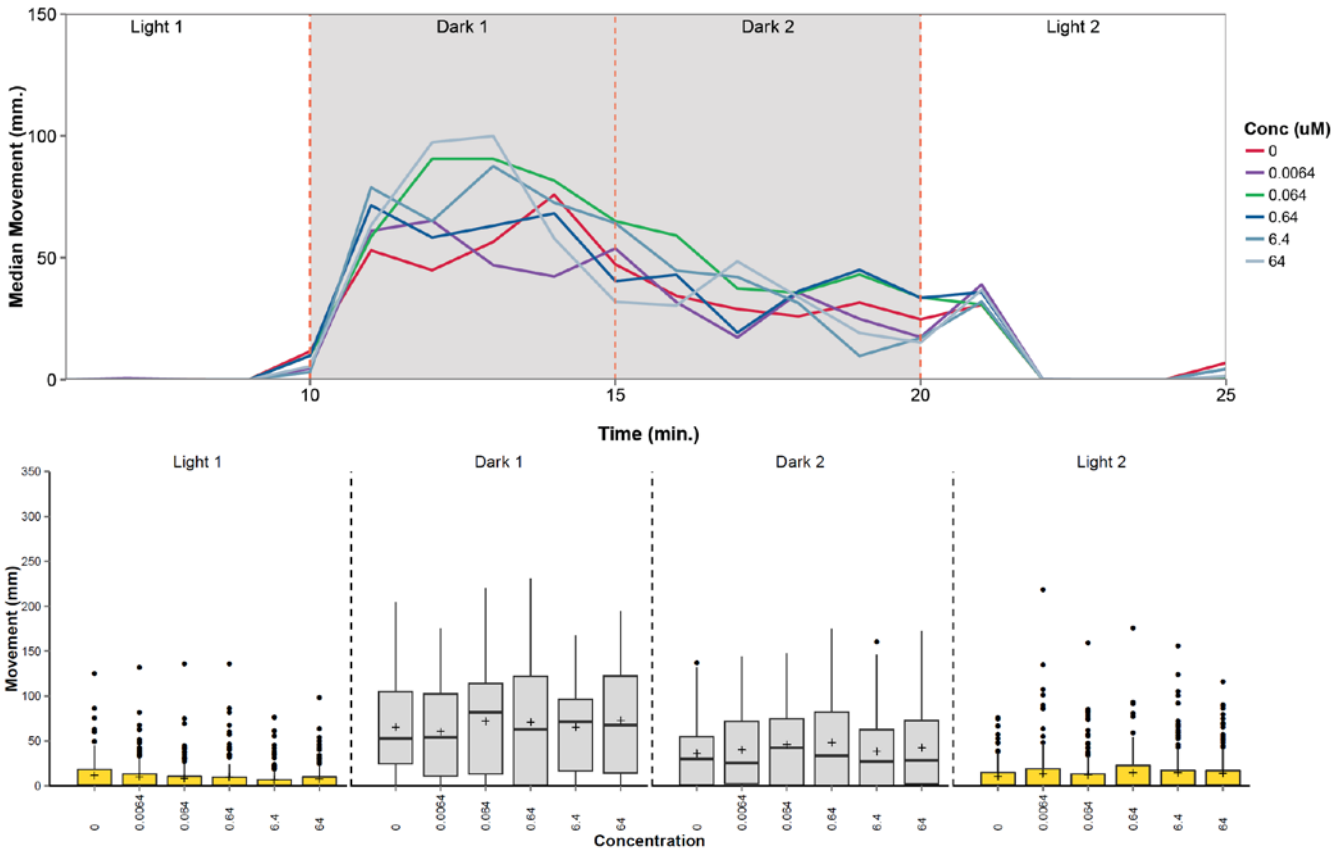
**DE-71: Zebrafish larvae PMR assay results at 120 hpf**



**DE-79: Zebrafish Mortality and Malformations at 24 and 120 hpf**



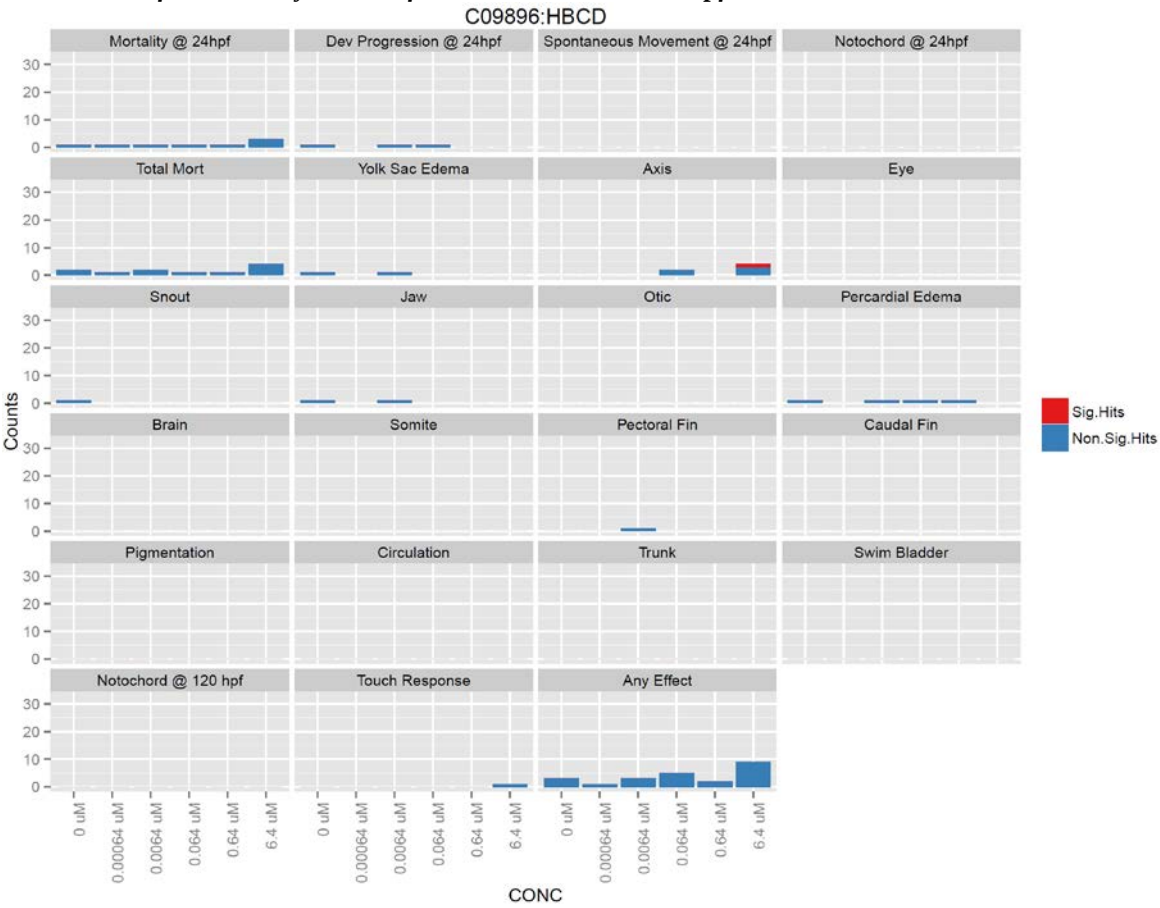
DE-79: Zebrafish larvae PMR assay results at 120 hpf



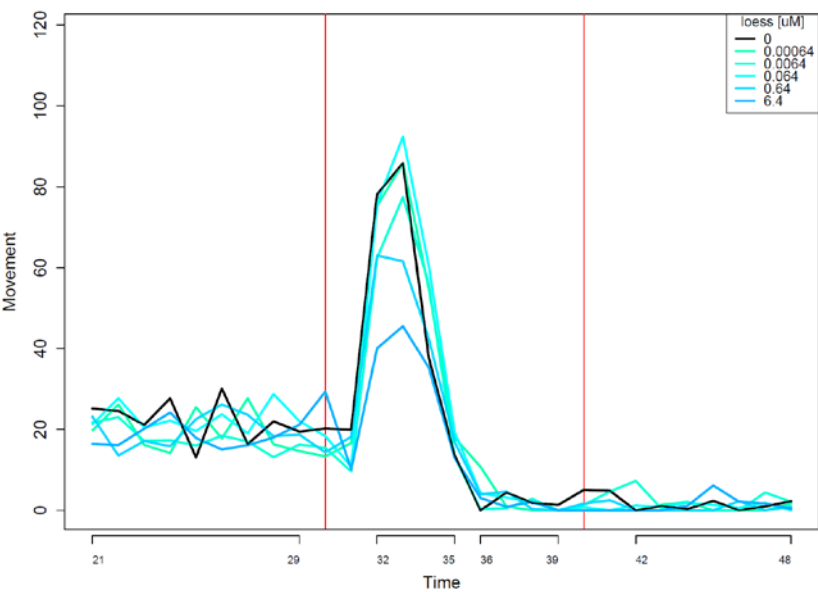
7. OTHER BROMINATED FLAME RETARDANTS

Hexabromocyclododecane (Commercial Mixture; HBCD)

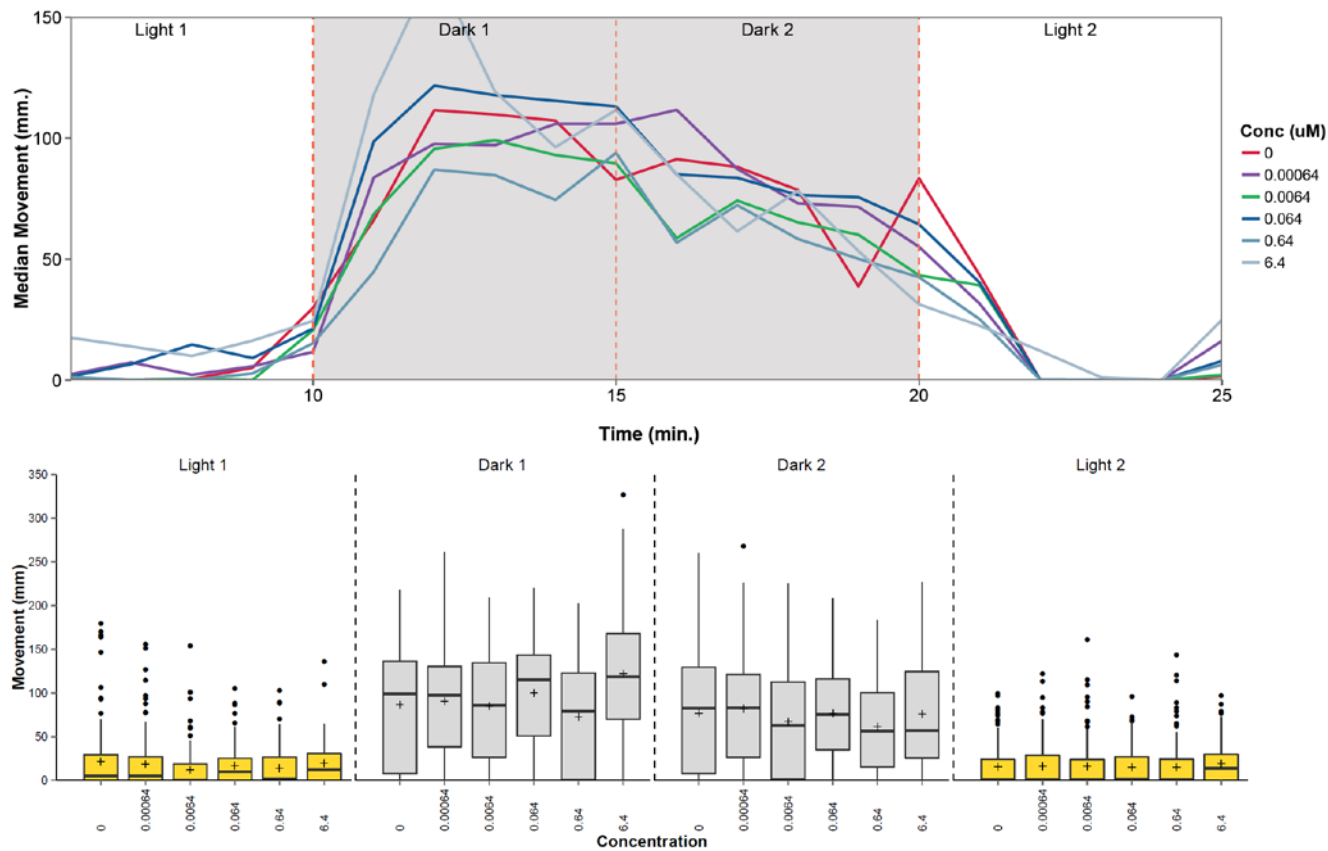
*HBCD: Zebrafish Mortality and Malformations at 24 and 120 hpf*



*HBCD: Zebrafish embryo PRAT results at 24 hpf*

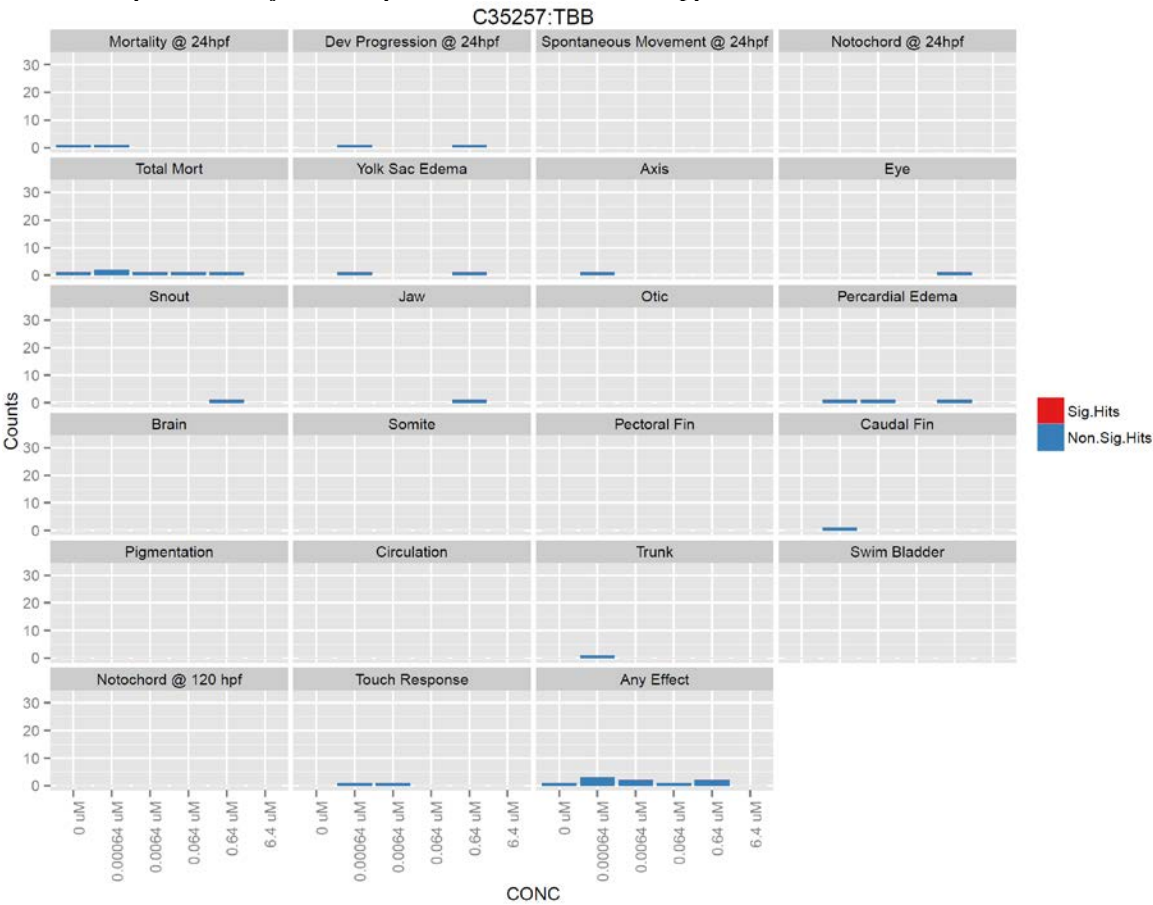


*HBCD: Zebrafish larvae PMR assay results at 120 hpf*

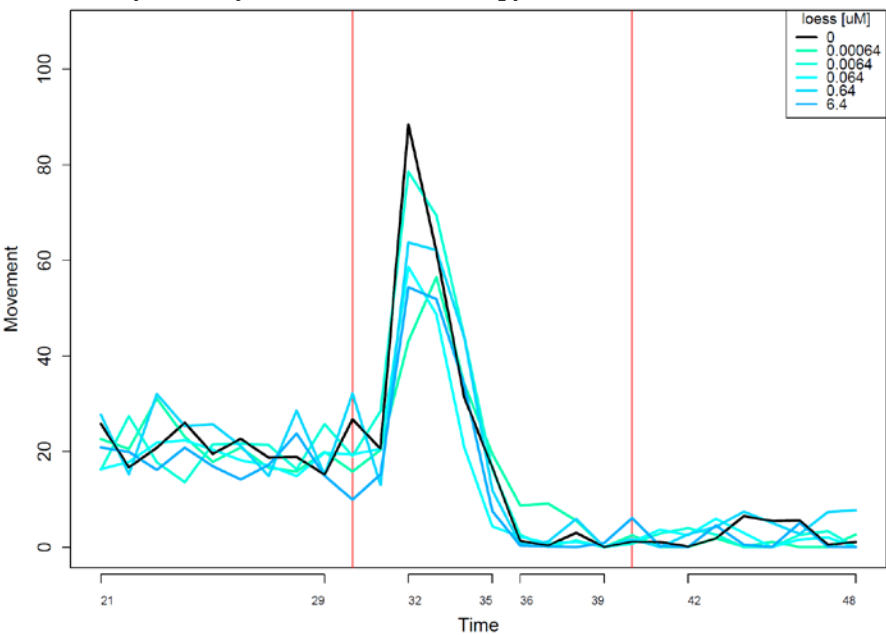


**2-ethylhexyl-tetrabromobenzoate (TBB)**

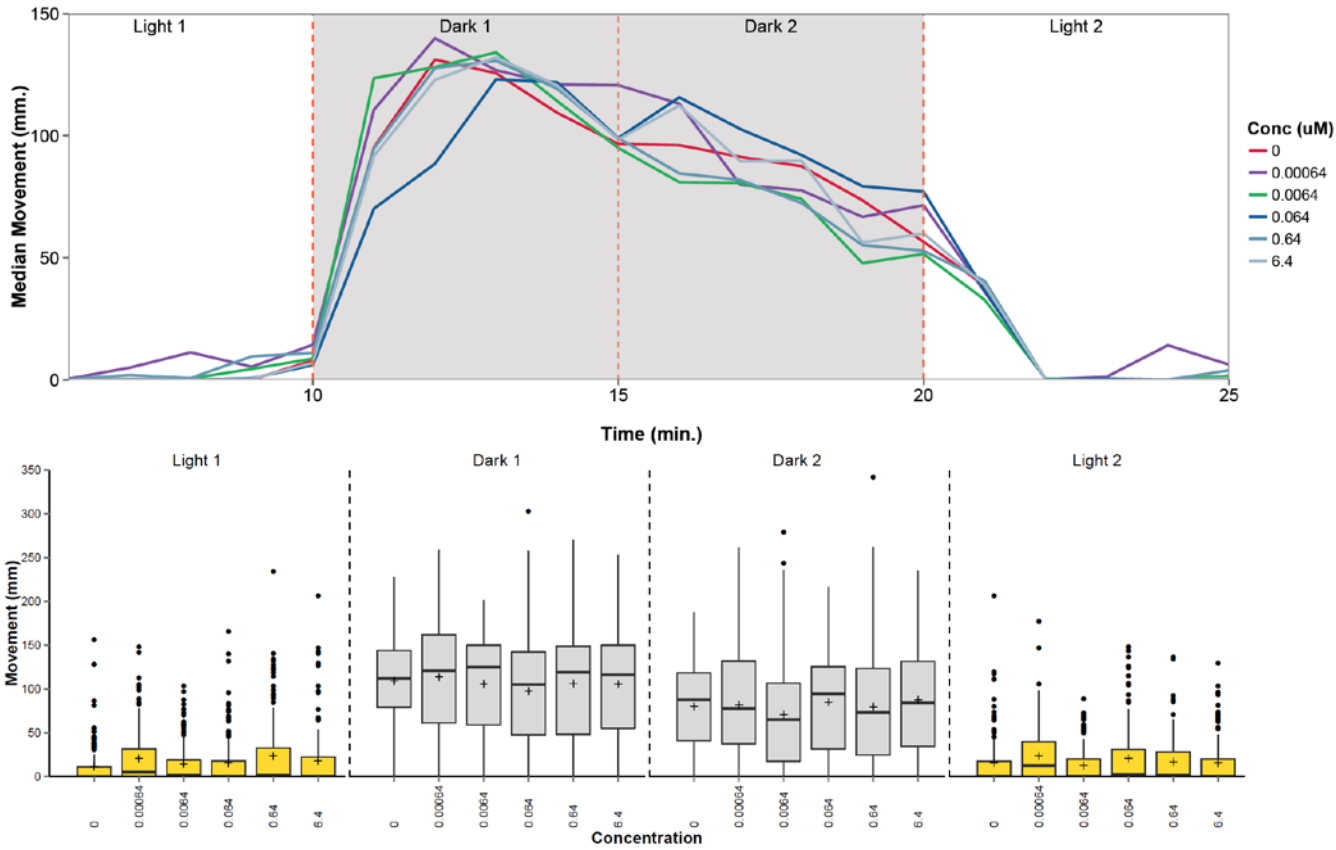
***TBB: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***TBB: Zebrafish embryo PRAT results at 24 hpf***

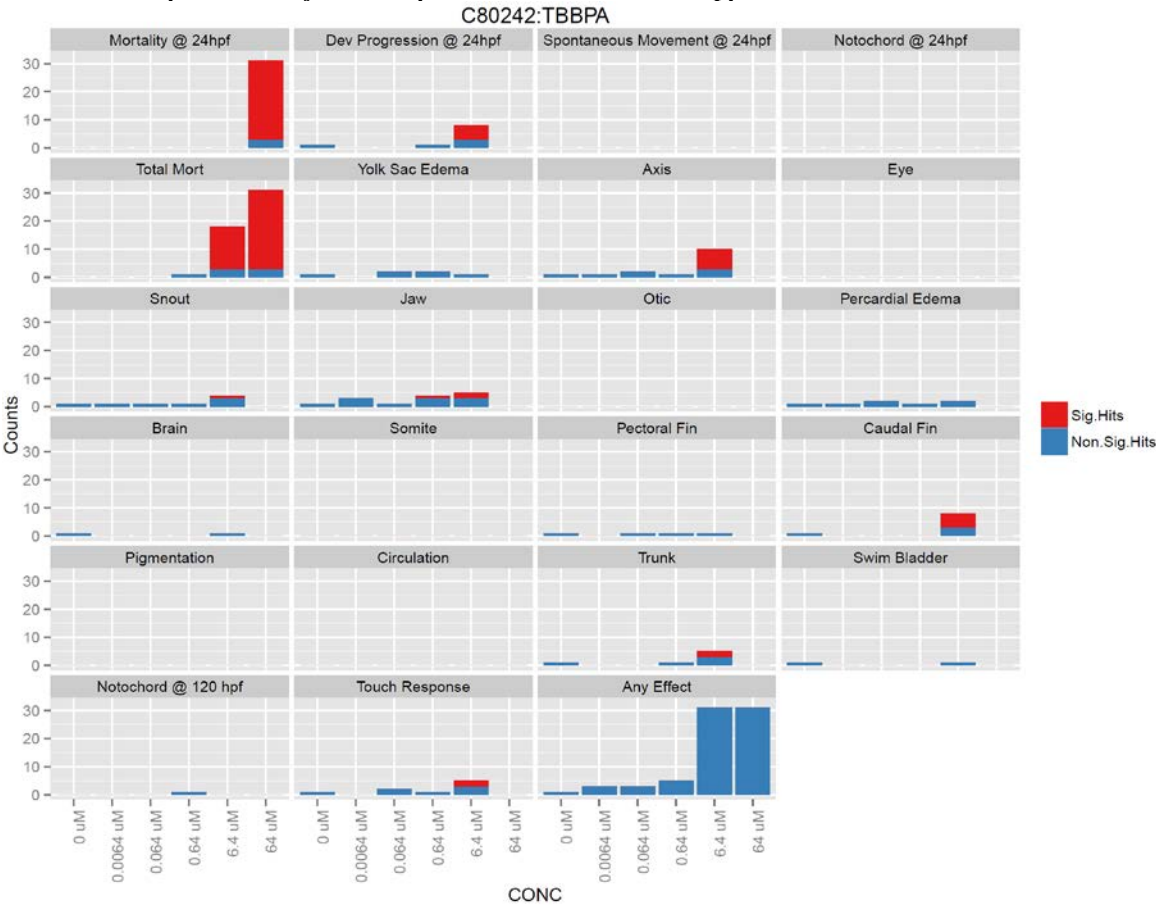


TBB: Zebrafish larvae PMR assay results at 120 hpf

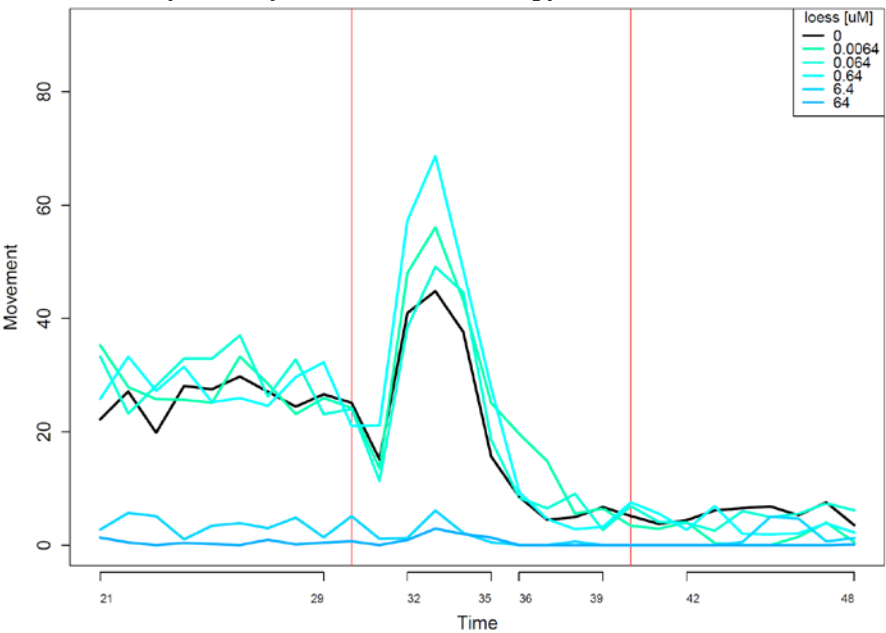


**3,3',5,5'-Tetrabromobisphenol A (TBBPA)**

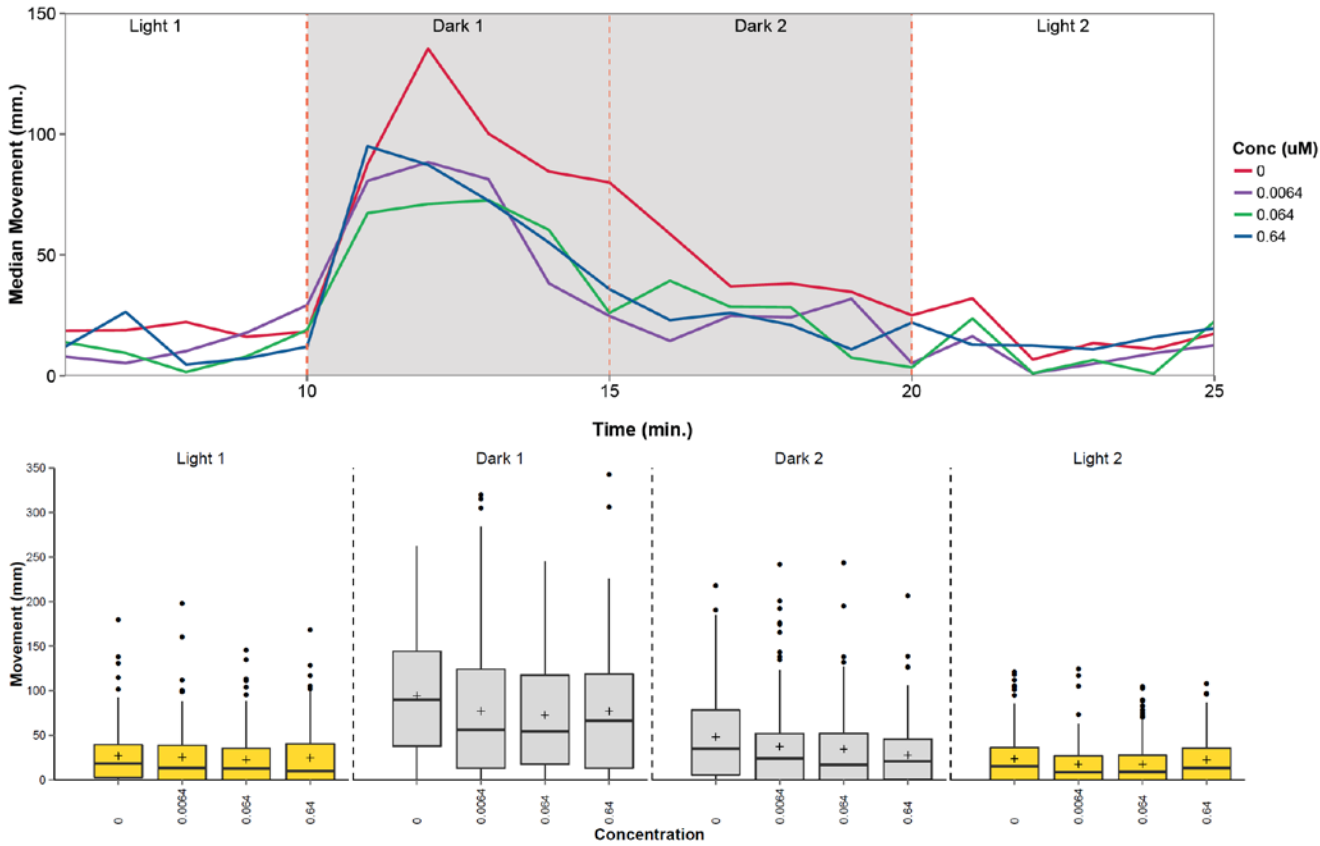
**TBBPA: Zebrafish Mortality and Malformations at 24 and 120 hpf**



**TBBPA: Zebrafish embryo PRAT results at 24 hpf**



TBBPA: Zebrafish larvae PMR assay results at 120 hpf

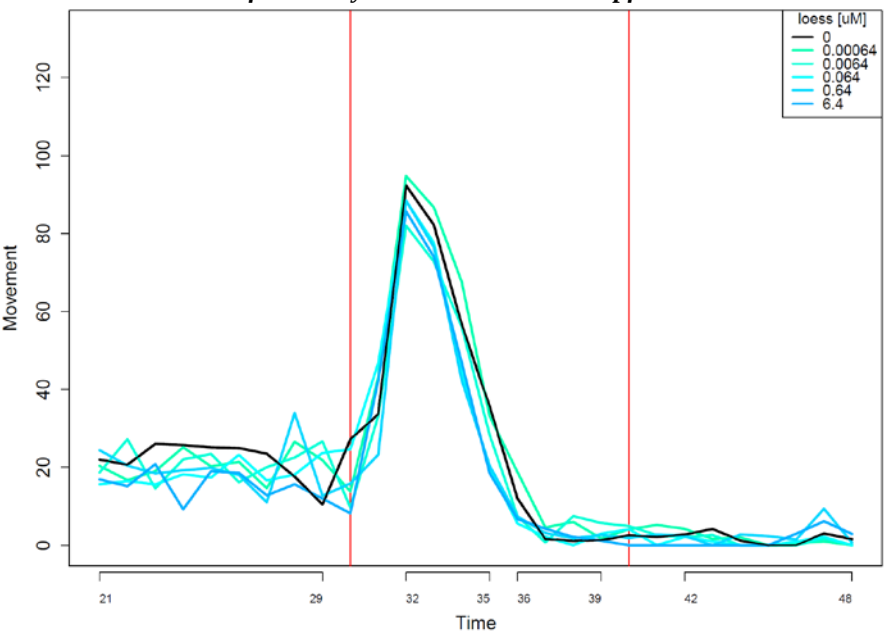


**Tetrabromobisphenol A-2,3-dibromopropyl ether (TBBPA-DBPE)**

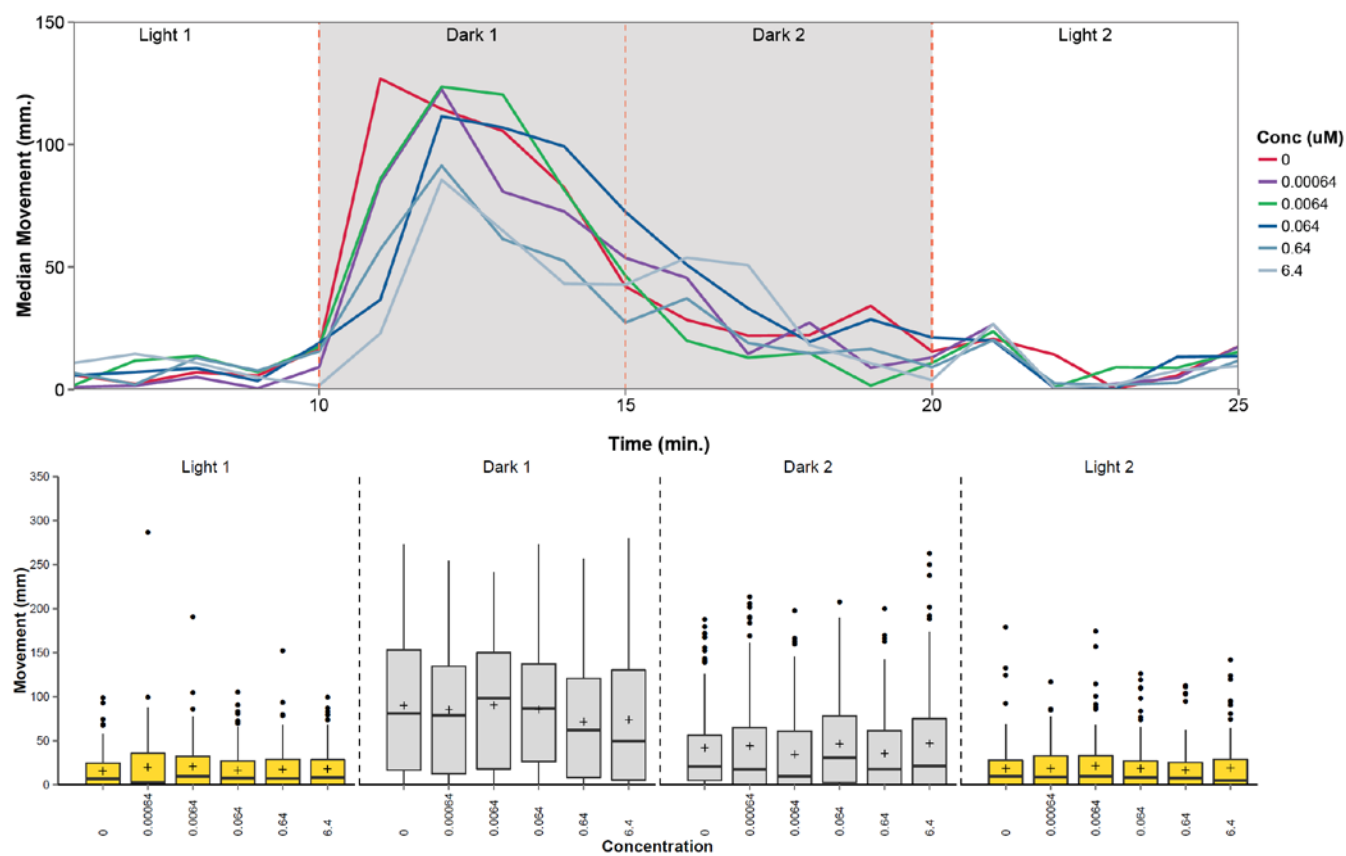
***TBBPA-DBPE: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***TBBPA-DBPE: Zebrafish embryo PRAT results at 24 hpf***

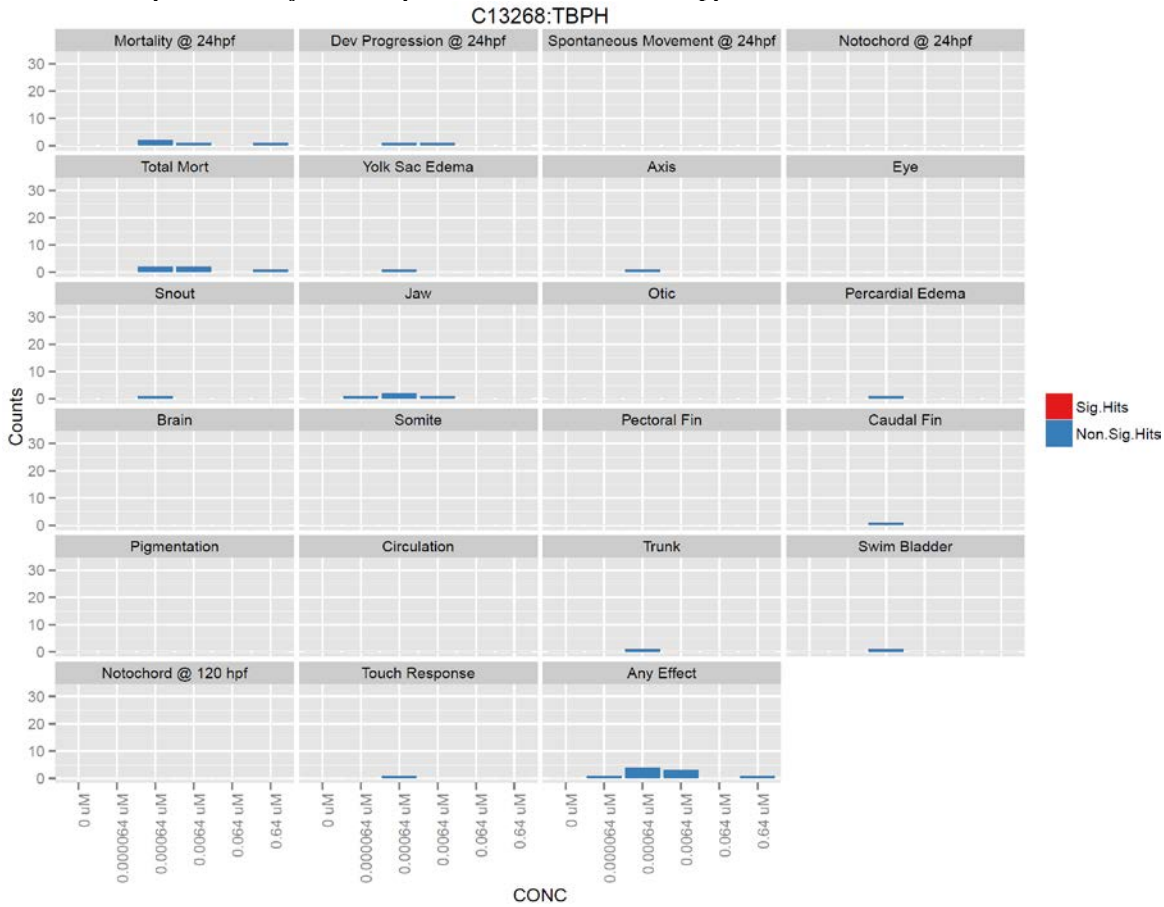


**TBBPA-DBPE: Zebrafish larvae PMR assay results at 120 hpf**

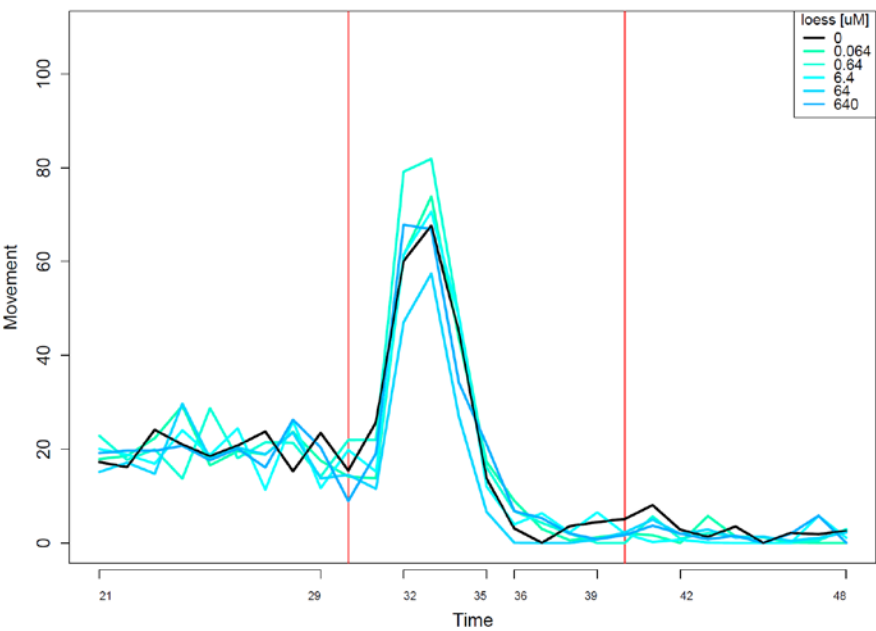


**Bis(2-ethylhexyl)-tetrabromophthalate (TBPH)**

***TBPH: Zebrafish Mortality and Malformations at 24 and 120 hpf***



***TBPH: Zebrafish embryo PRAT results at 24 hpf***



**TBPH: Zebrafish larvae PMR assay results at 120 hpf**

