United States
Environmental Protection Agency

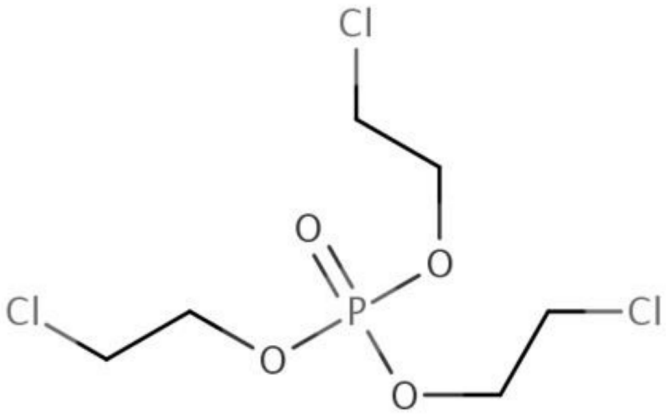
December 2023
Office of Chemical Safety and
Pollution Prevention

Draft Risk Evaluation for Tris(2-chloroethyl) phosphate (TCEP)

Systematic Review Supplemental File:

Data Quality Evaluation Information for Environmental Hazard

CASRN: 115-96-8



December 2023

This supplemental file contains information regarding the data quality evaluation results relevant to the characterization of environmental hazard for the Draft Risk Evaluation for Tris(2-chloroethyl) phosphate (TCEP). EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '2021 Draft Systematic Review Protocol'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Draft Risk Evaluation for Tris(2-chloroethyl) Phosphate (TCEP) - Systematic Review Protocol*.

Different data quality evaluation forms were used depending on the organism as described in the PECO statement in Appendix H.5.7 of the 2021 Draft Systematic Review Protocol. Each health outcome was evaluated independently within a given reference, therefore each reference may have more than one overall quality determination (OQD) to more appropriately reflect the quality of each health outcome and the respective hazard endpoints as described by the study authors. Some data evaluation forms have general additional comments presented adjacent to the OQD to add further context. No OQD is determined for each reference as a whole, if it contains data from more than one evidence stream. The table of contents lists references based on chemical, broad habitat (*e.g.*, aquatic, terrestrial), taxa, taxonomic group, exposure duration, and health outcome (*e.g.*, mortality) categories relevant to the endpoint being evaluated.

Table of Contents

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	Low	Biological response was difficult to interpret. Figure 1 reported affected embryos, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal, and end of test concentration on day four were not reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study did not report an acclimation period.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Percentage of altered and dead embryos wasused for Effective Concentration 50% (EC50) and Lethal Concentration 50% (LC50) calculations applying a nonlinear regression test (sigmoidal dose-response curve) using the GraphPad Prism (GraphPad Software).
	Metric 22:	Reporting of Data	Low	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	None			

Overall Quality Determination High

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	Low	Biological response was difficult to interpret. Figure 1 reported mortality, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Only initial nominal exposure concentrations were reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	10 min acclimation period was reported prior to behavior assessment (locomotor activity).
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The mean of the total distance moved by embryos in each group asmeasured in two-minute time bins and treated versus control groups were compared using unpaired Student's t-test.
	Metric 22:	Reporting of Data	Low	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	None			

Overall Quality Determination High

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Hepatic/Liver
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	High	Biological response was difficult to interpret. Figure 1 reported affected embryos, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal, and end of test concentration on day four were not reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study did not report an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Hepatic/Liver
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Percentage of altered and dead embryos was used for Effective Concentration 50% (EC50) and Lethal Concentration 50% (LC50) calculations applying a nonlinear regression test (sigmoidal dose-response curve) using the GraphPad Prism (GraphPad Software).
	Metric 22:	Reporting of Data	High	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	High	Biological response was difficult to interpret. Figure 1 reported affected embryos, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal, and end of test concentration on day four were not reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study did not report an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Percentage of altered and dead embryos was used for Effective Concentration 50% (EC50) and Lethal Concentration 50% (LC50) calculations applying a nonlinear regression test (sigmoidal dose-response curve) using the GraphPad Prism (GraphPad Software).
	Metric 22:	Reporting of Data	High	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	High	Biological response was difficult to interpret. Figure 1 reported affected embryos, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal, and end of test concentration on day four were not reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study did not report an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Percentage of altered and dead embryos was used for Effective Concentration 50% (EC50) and Lethal Concentration 50% (LC50) calculations applying a nonlinear regression test (sigmoidal dose-response curve) using the GraphPad Prism (GraphPad Software).
	Metric 22:	Reporting of Data	High	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Tris(2-chloroethyl) phosphate (TCEP), CASRN 115-96-8
	Metric 2:	Test Substance Source	High	Supplied by Sigma-Aldrich, Lot number SZBE090XV
	Metric 3:	Test Substance Purity	High	Purity = 95.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Vehicle/Solvent control (0.5% DMSO) and positive controls were used. No negative control reported. No mortality was reported in the vehicle control so lack of negative control is expected to have minimal impact on the results.
	Metric 5:	Negative Control Response	High	Biological response was difficult to interpret. Figure 1 reported affected embryos, but not for TCEP and based on other examples, "affected embryos" was near 10% with no mortality reported.
	Metric 6:	Randomized Allocation	Low	Organism allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study used well plates for experiments. Covering of well plates were not reported. Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure solutions were renewed on day two. However, remaining concentration in well plate prior to renewal, and end of test concentration on day four were not reported.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure group dosage and spacing were appropriate following a dose-range finding study.
	Metric 12:	Testing at or Below Solubility Limit	High	No indications were reported that TCEP concentration were above solubility limit. The study reported precipitation of other chemicals tested.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Embryos and life stages were adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study did not report an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of replicates were not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Alzualde, A., Behl, M., Sipes, N. S., Hsieh, J. H., Alday, A., Tice, R. R., Paules, R. S., Muriana, A., Quevedo, C. (2018). Toxicity profiling of flame retardants in zebrafish embryos using a battery of assays for developmental toxicity, neurotoxicity, cardiotoxicity and hepatotoxicity toward human relevance. Neurotoxicology and Teratology 7040-50.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5164137

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environment were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment were consistent among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Percentage of altered and dead embryos was used for Effective Concentration 50% (EC50) and Lethal Concentration 50% (LC50) calculations applying a nonlinear regression test (sigmoidal dose-response curve) using the GraphPad Prism (GraphPad Software).
	Metric 22:	Reporting of Data	High	Data were not clearly presented or described, but calculated endpoints were presented in the text and discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical substance was identified as the organophosphate flame retardant, TCEP.
	Metric 2:	Test Substance Source	High	The test substance was obtained from a reputable chemical manufacturer. The deuterated TCEP internal standards were synthesized in a single lab.
	Metric 3:	Test Substance Purity	High	The test substance had a 97% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	The metabolism study compared 1 day post fertilization and 5 day post fertilization parent chemical to metabolites in embryos and larvae. No negative control reported.
	Metric 5:	Negative Control Response	Uninformative	The study evaluated parent chemical to metabolite at 24 hours in embryos to levels in larvae at 5 days exposure. No negative control was used.
	Metric 6:	Randomized Allocation	Low	embryos were viewed under a dissecting microscope and only healthy embryos with intact chorions were selected for use in experiments. This is a supplemental metabolite study in zebrafish embryos.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details were sufficient to indicate consistency of exposure over 24 hour period for embryos.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Embryos were exposed to 1 uM solution of TCEP (in 10% Hanks' balanced salt solution) in glass petri dishes for 24 hours.
	Metric 10:	Exposure Duration and Frequency	High	The 24-hour exposure was appropriate for this metabolism study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposures were to a single concentration in embryos to evaluate metabolism, which was later compared with 5-day old larva metabolism.
	Metric 12:	Testing at or Below Solubility Limit	High	This was a metabolism study with a single concentration (which was below the water solubility limit of TCEP).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The test species is an established model for toxicity testing, but the authors did not report the the source of the zebrafish used in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Controls were not used for the metabolism study, and the authors referenced Padilla et al., 2011 for details on acclimatization and pretreatment.

Continued on next page . . .

...continued from previous page

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	This embryo metabolism test used 75-95 embryos per petri dish with 3 replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The embryos were collected from breeding tanks, washed and bleached, and healthy embryos were maintained in glass petri dishes maintained at 26C.
	Metric 17:	Outcome Assessment Methodology	High	The assessment methodology assessed metabolism after 24 hours of exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors did not report any variations in environmental conditions or other factors that could impact results.
	Metric 20:	Outcomes Unrelated to Exposure	High	The authors selected for healthy, viable embryos so that there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Tissue concentrations of the parent and primary metabolites were averaged, along with a two-way ANOVA and Sidak multiple comparisons for both the embryo and larval metabolism tests.
	Metric 22:	Reporting of Data	High	Data for the metabolism study for embryos and larva were presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes for the metabolism study.

Additional Comments: Metabolism study for zebrafish embryos accompanied by the larval metabolism study.

Overall Quality Determination Uninformative

Study Citation:	Du, Z., Wang, G., Gao, S., Wang, Z. (2015). Aryl organophosphate flame retardants induced cardiotoxicity during zebrafish embryogenesis: by disturbing expression of the transcriptional regulators. Aquatic Toxicology 16125-32.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB Strain; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4290535

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	toxicant identified by acronym and CAS number
	Metric 2:	Test Substance Source	Low	Sigma"Aldrich was the supplier, not analytically verified
	Metric 3:	Test Substance Purity	High	purity reported as 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	suitable solvent control was used
	Metric 5:	Negative Control Response	Medium	author reported strictly following OECD guidelines which, if valid, indicates that control survival was >=90%
	Metric 6:	Randomized Allocation	Low	no mention of randomization reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	daily renewal is adequate
	Metric 8:	Consistency of Exposure Administration	Medium	not enough details provided to warrant a high rating
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	High	followed oecd guidelines for acute tests
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was just stated that seven concentrations were used per chemical. There was no information on the test concentration used.
	Metric 12:	Testing at or Below Solubility Limit	High	EC50 was well below solubility limit
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented and met OEECD guidelines
	Metric 14:	Acclimatization and Pretreatment Conditions	High	reasonably well documented, seemed adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	followed OECD guidelines for organism number
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	enough documentation to verify adequacy
	Metric 17:	Outcome Assessment Methodology	High	simple and consistent methods used

Continued on next page . . .

...continued from previous page

Study Citation:	Du, Z., Wang, G., Gao, S., Wang, Z. (2015). Aryl organophosphate flame retardants induced cardiotoxicity during zebrafish embryogenesis: by disturbing expression of the transcriptional regulators. Aquatic Toxicology 16125-32.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB Strain; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4290535

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	methods used to quantify pericardial edema were not well documented
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no variables were reported in the study
	Metric 20:	Outcomes Unrelated to Exposure	High	no unexpected outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	methods of calculating EC50s were reported
	Metric 22:	Reporting of Data	High	clear reporting of EC50s
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	Du, Z., Wang, G., Gao, S., Wang, Z. (2015). Aryl organophosphate flame retardants induced cardiotoxicity during zebrafish embryogenesis: by disturbing expression of the transcriptional regulators. Aquatic Toxicology 16125-32.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB Strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4290535

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	toxicant identified by acronym and CAS number
	Metric 2:	Test Substance Source	Low	Sigma"Aldrich was the supplier, not analytically verified
	Metric 3:	Test Substance Purity	High	purity reported as 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	suitable solvent control was used
	Metric 5:	Negative Control Response	Medium	author reported strictly following OECD guidelines which, if valid, indicates that control survival was >=90%
	Metric 6:	Randomized Allocation	Low	no mention of randomization reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	daily renewal is adequate
	Metric 8:	Consistency of Exposure Administration	Medium	not enough details provided to warrant a high rating
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	High	followed oecd guidelines for acute tests
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was just stated that seven concentrations were used per chemical. There was no information on the test concentration used
	Metric 12:	Testing at or Below Solubility Limit	High	LC50 was well below solubility limit
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented and met OEECD guidelines
	Metric 14:	Acclimatization and Pretreatment Conditions	High	reasonably well documented, seemed adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	followed OECD guidelines for organism number
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	enough documentation to verify adequacy
	Metric 17:	Outcome Assessment Methodology	High	simple and consistent methods used
	Metric 18:	Consistency of Outcome Assessment	High	simple and consistent methods used

Continued on next page . . .

. . . continued from previous page

Study Citation:	Du, Z., Wang, G., Gao, S., Wang, Z. (2015). Aryl organophosphate flame retardants induced cardiotoxicity during zebrafish embryogenesis: by disturbing expression of the transcriptional regulators. Aquatic Toxicology 16125-32.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB Strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4290535

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no variables were reported in the study
	Metric 20:	Outcomes Unrelated to Exposure	High	no unexpected outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	methods of calculating LC50s were reported
	Metric 22:	Reporting of Data	High	clear reporting of LC50s
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	Jarema, K. A., Hunter, D. L., Shaffer, R. M., Behl, M., Padilla, S. (2015). Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicology and Teratology 52(Pt B):194-209.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4180931

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Well documented in supplemental data and table 1. IUPAC name and structure provided.
	Metric 2:	Test Substance Source	Low	NTP is listed as supplier, Sigma-Aldrich as the manufacturer but no analytical verification was reported
	Metric 3:	Test Substance Purity	High	Information is in supplemental data Table 1, purity of 99% reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	DMSO vehicle control was used as negative control, and chlorpyrifos or heptachlor were used as positive controls.
	Metric 5:	Negative Control Response	Low	No report on survival or malformation rate for controls, no comparison between clean and solvent controls were reported.
	Metric 6:	Randomized Allocation	Medium	Dose groups were randomized. "The location of each chemical concentration was randomized on the stock plate (96 well glass plate which mirrored the dosing on the experimental plate containing the embryos/larvae), and therefore the dose groups on the experimental plate were also randomized."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system was explained in detail.
	Metric 8:	Consistency of Exposure Administration	High	Method for administering the test substance was reported in detail, and was consistent across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test substance concentrations were not analytically verified. Wells were covered and sealed with Parafilm to minimize evaporation.
	Metric 10:	Exposure Duration and Frequency	High	durations were based on other studies
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Five concentrations were tested, covering an adequate range for a response. A range finding study was conducted to set the highest dose that would not cause any acute effects or lethality that would confound the behavioral assessment.
	Metric 12:	Testing at or Below Solubility Limit	Medium	No indication given for how solvent concentrations were chosen.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"Wild type adult zebrafish (Danio rerio), undefined, outbred stock originally obtained from Aquatic Research Organisms, Hampton, NH, 03842 and EkkWill Waterlife Resources, Ruskin, FL 33575 were" used for egg collection. Housing conditions for adult zebrafish and procedure for collecting eggs was described in detail.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Jarema, K. A., Hunter, D. L., Shaffer, R. M., Behl, M., Padilla, S. (2015). Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicology and Teratology 52(Pt B):194-209.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4180931

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Testing paradigm began with a 20 minute acclimation phase; all control and test organisms were subjected to the same pretreatment conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number tested at each concentration were reported – 24 larvae per dose.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions seemed adequate for embryo development, small quantities are difficult to assess.
	Metric 17:	Outcome Assessment Methodology	High	Well documented assessment procedure.
	Metric 18:	Consistency of Outcome Assessment	High	No deviations in assessment procedures were reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes reported downgraded because only normal larvae were assessed.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Well documented analysis methods.
	Metric 22:	Reporting of Data	High	All behavioral data was presented in a reasonable manner.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Downgraded because they only assessed normal larvae and none during the acclimation phase.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and structure presented in Figure 1.
	Metric 2:	Test Substance Source	High	Test substance source was identified and test substance purity was measured by MS and NMR techniques.
	Metric 3:	Test Substance Purity	High	Purity was measured at >98%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	DMSO controls were run concurrently.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentration spacing was adequate for the developmental experiment.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below reported water solubility limits and a solvent was employed to further aid solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organism source was not stated, otherwise the organisms were described adequately.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. 10 organisms per dish with 3 replicates per treatment level.
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	They state the embryos were reared in breeding water. There are uncertainties on whether this is the same water the adults were bred in (the assumption would be yes) and if the temperature is the same in the static test dishes as it is in the flow through tanks.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. Reduction in blood flow was measured by degree of circulation of red blood cells through a certain region of a trunk vessel.
	Metric 18:	Consistency of Outcome Assessment	High	Embryos were exposed at 72 hpf and assessed for reduction in blood flow at 96h using a 0-2 grading system under an inverted microscope.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Results on Blood Flow are presented in Supplemental Data Figure S2
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The cardiovascular outcome was chosen for this because it pertains to circulation of red blood cells and cardiac function.Data for this form are contained in Supplemental Figure S2. TCEP metabolite BCEP was also studied in this test.

Overall Quality Determination High

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical Structure was presented in Figure 1.
	Metric 2:	Test Substance Source	High	Test substance source was identified and test substance purity was measured by MS and NMR techniques.
	Metric 3:	Test Substance Purity	High	Purity was measured at >98%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	DMSO controls were run concurrently.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Random allocation was not detailed.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	concentration spacing was adequate for the developmental experiment.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below reported water solubility limits and a solvent was employed to further aid solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organism source was not stated, otherwise the organisms were described adequately.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. 10 organisms per dish with 3 replicates per treatment level.
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	They state the embryos were reared in breeding water. There are uncertainties on whether this is the same water the adults were bred in (the assumption would be yes) and if the temperature is the same in the static test dishes as it is in the flow through tanks.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. Pericardial edema was assessed at different treatment levels.
	Metric 18:	Consistency of Outcome Assessment	High	Embryos were exposed at 72 hpf and assessed for pericardial edema at 96h using a 0-2 grading system under an inverted microscope.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: Development/growth was selected because pericardial edema was being assessed as a morphological abnormality seen during an early life stagePE data are presented in Figure 4, Page 4/7. TCEP metabolite BCEP was also tested in this study.

Overall Quality Determination High

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical Structure was presented in Figure 1.
	Metric 2:	Test Substance Source	High	Test substance source was identified and test substance purity was measured by MS and NMR techniques.
	Metric 3:	Test Substance Purity	High	Purity was measured at >98%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	DMSO controls were run concurrently.
	Metric 5:	Negative Control Response	High	The biological response (mortality) of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Random allocation was not detailed.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	concentration spacing was adequate for the developmental experiment.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below reported water solubility limits and a solvent was employed to further aid solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organism source was not stated, otherwise the organisms were described adequately.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. 10 organisms per dish with 3 replicates per treatment level.
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Lee, J. S., Morita, Y., Kawai, Y. K., Covaci, A., Kubota, A. (2020). Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio. Chemosphere 246125738.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	7274629

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	They state the embryos were reared in breeding water. There are uncertainties on whether this is the same water the adults were bred in (the assumption would be yes) and if the temperature is the same in the static test dishes as it is in the flow through tanks.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. Pericardial edema was assessed at different treatment levels.
	Metric 18:	Consistency of Outcome Assessment	Medium	Embryos were exposed at 72 hpf and assessed Mortality at 96h. Specific criteria for mortality (ie, heart beat, response to stimuli, etc) was not reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the mortality observations reported in Fig 4 and Fig S2. TCEP exposures resulted in no mortality observations in the recorded data, however, mortality was reported as being recorded for all compounds in this study within the materials and methods.

Overall Quality Determination High

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Mortality assessment was described in the section titled "Developmental malformation evaluations" but authors did not report specific criteria for death (ie, movement, heartbeat, color, etc.).
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"Data collection was undertaken using a custom barcoding and tracking system (Zebrafish Acquisition and Analysis Program) to facilitate reliable management of the large amounts of data collected. Statistical analyses were performed using R code with testing methodologies used by Truong et al. (2014) to evaluate developmental toxicity of chemicals under the ToxCast program(RCoreTeam, 2014; Truong et al., 2014). Briefly, a binomial testwas performed that calculated lowest effect levels (LELs) foreach endpoint to identify incidences that exceeded a significantthreshold above controls. This test was preferable to a logisticregression as it accounted for the observed nonmonotonicity offlame retardant toxicity."
	Metric 22:	Reporting of Data	High	All counts of data per treatment and control group are represented in the supplemental data for each compound and time point (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for Mortality assessment at 24 hpf for TCEP. The specific data and statistical significance for each compound are located within the supplemental PDF.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric	Rating	Comments
Overall Quality Determination		**High**	

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Movement assays for embryos (24 hpf) and larvae (120 hpf) were described in detail on page 7 of 19.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Embryo movement: "Specifically, overall patterns of activity within each cycle interval (ie, baseline, excitation, refractory) were compared with those in vehicle controls by (1) estimating the 50% peak difference from controls in either direction and (2) performing a Kolmogorov"Smirnov test that compared the empirical cumulative distribution function between chemical treatments and controls. A Bonferroni-corrected p-value threshold of .01 (0.05/5 treatments".01) was used to determine statistical significance."Larval Movement: "As larval activity did not meet parametric assumptions of normality, Kruskal"Wallis analyses of variance and Dunn"s multiple comparison post tests were used to compare median locomotor activity per minute in treatment versus controls in each of the 5-min light/dark phases."
	Metric 22:	Reporting of Data	High	All raw data across time for embryo and larval movement is reported in the supplemental notes (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for embryo movement assessment at 24 hpf for TCEP.

Overall Quality Determination High

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods. 24 hour developmental assessments included: delays in developmental progression, notochord deformities, and altered spontaneous movements. 120 hour developmental assessments included: 17 developmental malformations, including yolk sac edema (YSE) and pericardial edema (PE); body axis (AXIS), trunk length (TRUN), caudal fin (CFIN), pectoral fin (PFIN), pigmentation (PIG), and somite (SOMI) deformities; eye (EYE), snout (SNOU), jaw (JAW), and otolith (OTIC) malformations; gross brain development (BRAIN); notochord (NC) and circulatory (CIRC) deformities; swim bladder presence and inflation (SWIM); and touch responses (TR).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.

Continued on next page . . .

. . . continued from previous page

Study Citation: Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path: Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome: Development/Growth
Chemical: Tris(2-chloroethyl) phosphate (TCEP)
HERO ID: 2953504

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Developmental assessment was described in the section titled "Developmental malformation evaluations". The specifc analysis is detailed in another publication that authors referenced as HERO ID 8591199
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	"Data collection was undertaken using a custom barcoding and tracking system (Zebrafish Acquisition and Analysis Program) to facilitate reliable management of the large amounts of data collected. Statistical analyses were performed using R code with testing methodologies used by Truong et al. (2014) to evaluate developmental toxicity of chemicals under the ToxCast program(RCoreTeam, 2014; Truong et al., 2014). Briefly, a binomial testwas performed that calculated lowest effect levels (LELs) foreach endpoint to identify incidences that exceeded a significantthreshold above controls. This test was preferable to a logisticregression as it accounted for the observed nonmonotonicity offlame retardant toxicity."This reference is HERO ID 8591199
	Metric 22:	Reporting of Data	High	All counts of data per treatment and control group are represented in the supplemental data for each compound and time point (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for developmental assessment at 24 hpf for TCEP. The specific data and statistical significance for each compound are located within the supplemental PDF.

Overall Quality Determination High

Study Citation:	Wang, G. W., Chen, H. Y., Du, Z. K., Li, J. H., Wang, Z. Y., Gao, S. X. (2017). In vivo metabolism of organophosphate flame retardants and distribution of their main metabolites in adult zebrafish. Science of the Total Environment 590:50-59.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5166352

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name, CAS# and structure. Details provided in Table S1.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The use of a control is implied in order to calculate 96h LC 50 according to OECD 203 protocol.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided no details on exposure media preparation for the mortality experiment.
	Metric 8:	Consistency of Exposure Administration	Low	The study provided no details on exposure administration for the mortality experiment.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported (96 h) and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and spacing of exposure levels used to determine 96h LC 50.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a reliable source but specifics such as average weight were not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate and whether differences occurred between control and exposed populations.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wang, G. W., Chen, H. Y., Du, Z. K., Li, J. H., Wang, Z. Y., Gao, S. X. (2017). In vivo metabolism of organophosphate flame retardants and distribution of their main metabolites in adult zebrafish. Science of the Total Environment 590:50-59.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5166352

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was stated that the experiment was conducted according to OECD 203 but no other details were provided.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were summarized in the text as an LC50 value.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.

Additional Comments: LC 50 values were determined according to OECD 203 but no details (regarding test media preparation, number of exposure groups, control response, number of organisms and replicates, mortality data for each treatment group, environmental conditions, etc.) were provided. The main focus of the paper was to study in vivo metabolism of TCEP and the LC50 values were used to set up the exposure groups for the main experiment.

Overall Quality Determination Uninformative

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified by name and CAS #
	Metric 2:	Test Substance Source	Low	Test substance source was reported as Sigma-Aldrich, but it did not appear to be analyzed by the performing laboratory
	Metric 3:	Test Substance Purity	High	Test substance purity was 98.95%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	All treated groups were compared to a DMSO vehicle controls.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported. The biological response of the positive TOCP control was reported though.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	DMSO solvent was used in the preparation of the test media stock solutions, but little other information was provided on preparation. The experimental system was 96 well plates with one egg per plate.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. 5 day exposure with one day in Hanks' buffer before assessment.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type–5 day exposure with 1 day in Hanks' buffer only.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	A concentration range of 2.12uM to 120uM was reported with a concentration interval of about 0.25log10 units. The exact number of exposure groups was not reported.
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO solvent concentration was appropriate at 0.1% (v/v)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized

Continued on next page . . .

. . . continued from previous page

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect. 1 embryo per well/test concentration with at least 4 replicates of each concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. Environmental conditions of breeding adults was reported, but conditions were not reported for embryos.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups–assessment was performed 6dpf after 1 day in Hanks' buffer without test substance
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment was reported, but no data regarding control performance was recorded.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified by name and CAS #
	Metric 2:	Test Substance Source	Low	Test substance source was reported as Sigma-Aldrich, but it did not appear to be analyzed by the performing laboratory
	Metric 3:	Test Substance Purity	High	Test substance purity was 98.95%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	All treated groups were compared to DMSO vehicle controls.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported. The biological response of the positive TOCP control was reported though.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	DMSO solvent was used in the preparation of the test media stock solutions, but little other information was provided on preparation. The experimental system was 96 well plates with one egg per plate.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. 5 day exposure with one day in Hanks' buffer before assessment.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type–5 day exposure with 1 day in Hanks' buffer only.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	A concentration range of 2.12uM to 120uM was reported with a concentration interval of about 0.25log10 units. The exact number of exposure groups was not reported.
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO solvent concentration was appropriate at 0.1% (v/v)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect. 1 embryo per well/test concentration with at least 4 replicates of each concentration.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. Environmental conditions of breeding adults was reported, but conditions were not reported for embryos.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups–assessment was performed 6dpf after 1 day in Hanks' buffer without test substance
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis did not appear to be performed on mortality alone. Just percent mortality was reported. Furthermore, death and non-hatching were grouped together with developmental toxicity.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment was reported, but no data regarding control performance was recorded.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability in mortality was not provided.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical substance was identified as the organophosphate flame retardant, TCEP.
	Metric 2:	Test Substance Source	High	The test substance was obtained from a reputable chemical manufacturer.
	Metric 3:	Test Substance Purity	High	The test substance had a 97% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a negative control with 10% HBSS and 0.4% DMSO.
	Metric 5:	Negative Control Response	High	There were no developmental abnormalities among control fish.
	Metric 6:	Randomized Allocation	Medium	Embryos were collected from multiple breeding tanks and pooled, then 1 embryo/well in 96-well plates used for exposures.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details were sufficient to indicate consistency of exposure over 6 days post-fertilization.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The researchers did not measure exposure concentrations, but renewed the non-volatile test substance daily.
	Metric 10:	Exposure Duration and Frequency	Low	The 6-day exposures, while informative, do not qualify for early-life stage testing, which require 30-day post hatch exposures.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The test organisms were exposed to 1/2 log increments of TCEP concentrations ranging from 0.033-100uM.
	Metric 12:	Testing at or Below Solubility Limit	High	Testing was below the water solubility for TCEP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test species is an established model for toxicity testing, so although the authors did not report the the source of the zebrafish used in the study, results for controls indicate that this will not have a substantial impact on results.
	Metric 14:	Acclimatization and Pretreatment Conditions	N/A	The authors referenced Padilla et al., 2011 for details on acclimatization and pretreatment of the test organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The study used 8 fish/concentration in two replicates. While not adequate for OPPT TG 850.1400 for fish early life-stage testing, concentration-response relationships were observed and the number was sufficient for statistical analysis.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The larvae were maintained in glass petri dishes maintained at 26C.
	Metric 17:	Outcome Assessment Methodology	High	The assessment methodology assessed developmental abnormalities after 6 days of exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors did not report any variations in environmental conditions or other factors that could impact results.
	Metric 20:	Outcomes Unrelated to Exposure	High	The authors did not indicate differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were adequately described for the study.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes for the development study.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical substance was identified as the organophosphate flame retardant, TCEP.
	Metric 2:	Test Substance Source	High	The test substance was obtained from a reputable chemical manufacturer.
	Metric 3:	Test Substance Purity	High	The test substance had a 97% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a negative control with 10% HBSS and 0.4% DMSO.
	Metric 5:	Negative Control Response	High	There was no mortality among control fish.
	Metric 6:	Randomized Allocation	Medium	Embryos were collected from multiple breeding tanks and pooled, then 1 embryo/well in 96-well plates used for exposures.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details were sufficient to indicate consistency of exposure over 6 days post-fertilization.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The researchers did not measure exposure concentrations, but renewed the non-volatile test substance daily.
	Metric 10:	Exposure Duration and Frequency	Low	The 6-day exposures, while informative, do not qualify for early-life stage testing, which require 30-day post hatch exposures.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The test organisms were exposed to 1/2 log increments of TCEP concentrations ranging from 0.033-100uM.
	Metric 12:	Testing at or Below Solubility Limit	High	Testing was below the water solubility for TCEP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test species is an established model for toxicity testing, so although the authors did not report the the source of the zebrafish used in the study, results for controls indicate that this will not have a substantial impact on results.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors referenced Padilla et al., 2011 for details on acclimatization and pretreatment of the test organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The study used 8 fish/concentration in two replicates. While not adequate for OPPT TG 850.1400 for fish early life-stage testing, concentration-response relationships were observed and the number was sufficient for statistical analysis.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The larvae were maintained in glass petri dishes maintained at 26C.

Continued on next page . . .

...continued from previous page

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The assessment methodology assessed mortality after 6 days of exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors did not report any variations in environmental conditions or other factors that could impact results.
	Metric 20:	Outcomes Unrelated to Exposure	High	The authors did not indicate differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were adequately described for the study.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes for the mortality study.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical substance was identified as the organophosphate flame retardant, TCEP.
	Metric 2:	Test Substance Source	High	The test substance was obtained from a reputable chemical manufacturer. The deuterated TCEP internal standards were synthesized in a single lab.
	Metric 3:	Test Substance Purity	High	The test substance had a 97% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	The metabolism study compared 1 day post fertilization and 5 day post fertilization parent chemical to metabolites in embryos and larvae. No negative control reported.
	Metric 5:	Negative Control Response	Uninformative	The study evaluated parent chemical to metabolite at 24 hours in embryos to levels in larvae at 5 days exposure. No negative control was used.
	Metric 6:	Randomized Allocation	Low	embryos were viewed under a dissecting microscope and only healthy embryos with intact chorions were selected for use in experiments. This is a supplemental metabolite study in zebrafish embryos.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details were sufficient to indicate consistency of exposure over 5 days.
	Metric 9:	Measurement of Test Substance Concentration	Low	Larvae were exposed to 1 uM solution of TCEP in glass petri dishes for 5 days, without renewal or analytical monitoring.
	Metric 10:	Exposure Duration and Frequency	High	The 5-day exposure was appropriate for this metabolism study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposures were to a single concentration in larvae to evaluate metabolism, which was later compared with 1-day exposure in embryos.
	Metric 12:	Testing at or Below Solubility Limit	High	This was a metabolism study with a single concentration (which was below the water solubility limit of TCEP).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The test species is an established model for toxicity testing, but the authors did not report the the source of the zebrafish used in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Controls were not used for the metabolism study, and the authors referenced Padilla et al., 2011 for details on acclimatization and pretreatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The larval metabolism test had 3 replicates of 3 samples (20 fish per sample).

Continued on next page . . .

. . . continued from previous page

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The larvae were maintained in glass petri dishes maintained at 26C.
	Metric 17:	Outcome Assessment Methodology	High	The assessment methodology assessed metabolism after 5 days of exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors did not report any variations in environmental conditions or other factors that could impact results.
	Metric 20:	Outcomes Unrelated to Exposure	High	The authors did not indicate differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Tissue concentrations of the parent and primary metabolites were averaged, along with a two-way ANOVA and Sidak multiple comparisons for both the embryo and larval metabolism tests.
	Metric 22:	Reporting of Data	High	Data for the metabolism study for embryos and larva were presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes for the metabolism study.

Additional Comments: Metabolism study for zebrafish embryos accompanied by the larval metabolism study.

Overall Quality Determination Uninformative

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical substance was identified as the organophosphate flame retardant, TCEP.
	Metric 2:	Test Substance Source	High	The test substance was obtained from a reputable chemical manufacturer.
	Metric 3:	Test Substance Purity	High	The test substance had a 97% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a negative control with 10% HBSS and 0.4% DMSO.
	Metric 5:	Negative Control Response	High	The control larval swimming behavior appears acceptable.
	Metric 6:	Randomized Allocation	Medium	Embryos were extracted from multiple breeding tanks and pooled before cleaning and inspection for viability.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details were sufficient to indicate consistency of exposure over 6 days post-fertilization.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The researchers did not measure exposure concentrations, but renewed the non-volatile test substance daily.
	Metric 10:	Exposure Duration and Frequency	Low	The 6-day exposures, while informative, do not qualify for early-life stage testing, which require 30-day post hatch exposures.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The test organisms were exposed to 5 concentrations of 1/4 log increments of TCEP concentrations set below concentrations that resulted in mortality or developmental abnormalities.
	Metric 12:	Testing at or Below Solubility Limit	High	Testing was below the water solubility for TCEP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test species is an established model for toxicity testing, so although the authors did not report the the source of the zebrafish used in the study, results for controls indicate that this will not have a substantial impact on results.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors referenced Padilla et al., 2011 for details on acclimatization and pretreatment of the test organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The study used 6-12 fish/concentration with 2-4 replicates. While not adequate for OPPT TG 850.1400 for fish early life-stage testing, concentration-response relationships were observed and the number was sufficient for statistical analysis.
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., Stapleton, H. M. (2014). Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (Danio rerio). Toxicological Sciences 142(2):445-454.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3014520

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	The larvae were maintained in glass petri dishes maintained at 26C.
	Metric 17:	Outcome Assessment Methodology	High	The assessment methodology assessed changes in larval swimming behavior after 6 days of exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors did not report any variations in environmental conditions or other factors that could impact results.
	Metric 20:	Outcomes Unrelated to Exposure	High	The authors did not indicate differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were adequately described for the study.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes for the behavioral study.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Jarema, K. A., Hunter, D. L., Shaffer, R. M., Behl, M., Padilla, S. (2015). Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicology and Teratology 52(Pt B):194-209.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4180931

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Well documented in supplemental data and table 1. IUPAC name and structure provided.
	Metric 2:	Test Substance Source	Low	NTP is listed as supplier, Sigma-Aldrich as the manufacturer, purity was not analytically verified.
	Metric 3:	Test Substance Purity	High	Information is in supplemental data, listed as 99% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	DMSO vehicle control was used as negative control, and chlorpyrifos or heptachlor were used as positive controls.
	Metric 5:	Negative Control Response	Low	No report on survival or malformation rate for controls, no comparison between clean and solvent controls were reported.
	Metric 6:	Randomized Allocation	Medium	Dose groups were randomized. "The location of each chemical concentration was randomized on the stock plate (96 well glass plate which mirrored the dosing on the experimental plate containing the embryos/larvae), and therefore the dose groups on the experimental plate were also randomized."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system was explained in detail.
	Metric 8:	Consistency of Exposure Administration	High	Method for administering the test substance was reported in detail, and was consistent across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test substance concentrations were not analytically verified. Wells were covered and sealed with Parafilm to minimize evaporation.
	Metric 10:	Exposure Duration and Frequency	High	durations were based on other studies
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Five concentrations were tested, covering an adequate range for a response. A range finding study was conducted to set the highest dose that would not cause any acute effects or lethality that would confound the behavioral assessment.
	Metric 12:	Testing at or Below Solubility Limit	Medium	No indication given for how solvent concentrations were chosen.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"Wild type adult zebrafish (Danio rerio), undefined, outbred stock originally obtained from Aquatic Research Organisms, Hampton, NH, 03842 and EkkWill Waterlife Resources, Ruskin, FL 33575 were" used for egg collection. Housing conditions for adult zebrafish and procedure for collecting eggs was described in detail.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Jarema, K. A., Hunter, D. L., Shaffer, R. M., Behl, M., Padilla, S. (2015). Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicology and Teratology 52(Pt B):194-209.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4180931

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Treatments were reported as similar across all treatments.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number tested at each concentration were reported – 24 larvae per dose.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions seemed adequate for embryo development, small quantities are difficult to assess.
	Metric 17:	Outcome Assessment Methodology	High	Well documented assessment procedure.
	Metric 18:	Consistency of Outcome Assessment	High	No deviations in assessment procedures were reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes reported downgraded because only normal larvae were assessed.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Well documented analysis methods
	Metric 22:	Reporting of Data	High	All behavioral data was presented in a reasonable manner.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Downgraded because they only assessed normal larvae and none during the acclimation phase.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP identified by CAS number and name
	Metric 2:	Test Substance Source	Low	TCEP was not analytically verified
	Metric 3:	Test Substance Purity	Medium	97% purity reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	the solvent concentration of treatments wasn't reported
	Metric 5:	Negative Control Response	High	good survival (>90%) in controls
	Metric 6:	Randomized Allocation	Low	did not report random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	adequate test system and description
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies reported between treatments
	Metric 9:	Measurement of Test Substance Concentration	High	test concentrations were measured
	Metric 10:	Exposure Duration and Frequency	High	adequate duration to observe desired effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate number of exposure groups to assess outcome
	Metric 12:	Testing at or Below Solubility Limit	High	well within solubility of TCEP
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately characterized
	Metric 14:	Acclimatization and Pretreatment Conditions	High	consistent pre treatment across all concentrations
	Metric 15:	Number of Organisms and Replicates per Group	Medium	four replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	test water parameters except temperature were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	adequate to determine percent hatch and survival
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported

Continued on next page . . .

. . . continued from previous page

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methodology was adequate for this outcome
	Metric 22:	Reporting of Data	High	data reporting was reasonably clear
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported

Additional Comments: This evaluation form pertains to hatching rate of embryos following exposure to 0, 100, 500 or 2500 mg/L of TCEP.

Overall Quality Determination High

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Neurotoxicology
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP identified by CAS number and name
	Metric 2:	Test Substance Source	Low	TCEP was not analytically verified
	Metric 3:	Test Substance Purity	Medium	97% purity reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	the solvent concentration of treatments wasn't reported
	Metric 5:	Negative Control Response	High	good survival (>90%) in controls
	Metric 6:	Randomized Allocation	Low	did not report random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	adequate test system and description
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies reported between treatments
	Metric 9:	Measurement of Test Substance Concentration	High	test concentrations were measured
	Metric 10:	Exposure Duration and Frequency	Medium	adequate duration to observe desired effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	the number of exposure groups were adequate to assess this outcome
	Metric 12:	Testing at or Below Solubility Limit	High	well within solubility of TCEP
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately characterized
	Metric 14:	Acclimatization and Pretreatment Conditions	High	consistent pre treatment across all concentrations
	Metric 15:	Number of Organisms and Replicates per Group	Medium	four replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	test water parameters except temperature were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	enzyme and protein determination and gene transcription methods seemed sound
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Neurotoxicology
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methodology was adequate for this outcome
	Metric 22:	Reporting of Data	High	determinations of levels of significance seemed sound
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported

Additional Comments: This evaluation form pertains to mechanistic endpoints (neurotransmitter contents, AchE activity, gene transcription and protein expression) evaluated in zebrafish larvae after exposure to TCEP.

Overall Quality Determination High

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP identified by CAS number and name
	Metric 2:	Test Substance Source	Low	TCEP was not analytically verified
	Metric 3:	Test Substance Purity	Medium	97% purity reported for TCEP
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	the solvent concentration of treatments wasn't reported
	Metric 5:	Negative Control Response	High	good survival (>90%) in controls
	Metric 6:	Randomized Allocation	Low	did not report random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	adequate test system and description
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies reported between treatments
	Metric 9:	Measurement of Test Substance Concentration	High	test concentrations were measured
	Metric 10:	Exposure Duration and Frequency	High	adequate duration to observe desired effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate number of organisms to observe desired effects
	Metric 12:	Testing at or Below Solubility Limit	High	well within solubility of TCEP
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	adequately documented organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	consistent pre treatment across all concentrations
	Metric 15:	Number of Organisms and Replicates per Group	Medium	four replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	test water parameters except temperature were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	adequate measurements but excluding malformed organisms is concerning
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methodology seemed sound
	Metric 22:	Reporting of Data	High	data reporting was reasonably clear
	Metric 23:	Explanation of Unexpected Outcomes	High	adequate explanation of difference between light and dark response

Additional Comments: This evaluation form pertains to locomotor behavior measurement of zebrafish larvae.

Overall Quality Determination High

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP identified by CAS number and name
	Metric 2:	Test Substance Source	Low	TCEP was not analytically verified
	Metric 3:	Test Substance Purity	Medium	97% purity reported for TCEP
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	the solvent concentration of treatments wasn't reported
	Metric 5:	Negative Control Response	High	good survival (>90%) in controls
	Metric 6:	Randomized Allocation	Low	did not report random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	adequate test system and description
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies reported between treatments
	Metric 9:	Measurement of Test Substance Concentration	High	test concentrations were reported
	Metric 10:	Exposure Duration and Frequency	Medium	adequate duration to observe desired effects, not primary purpose of study
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate number of exposure groups to assess outcomes
	Metric 12:	Testing at or Below Solubility Limit	High	well within solubility of TCEP
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	adequate documentation of organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	consistent pre treatment across all concentrations
	Metric 15:	Number of Organisms and Replicates per Group	Medium	four replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	test water parameters except temperature were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	type of malformations not reported
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were noted
Domain 6: Confounding / Variable Control				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no unexpected variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methods were adequately explained
	Metric 22:	Reporting of Data	High	documentation of data reporting as adequate
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexplained outcomes were reported

Additional Comments: This evaluation form pertains to malformation rate of embryos following exposure to 0, 100, 500 or 2500 mg/L of TCEP.

Overall Quality Determination High

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP identified by CAS number and name
	Metric 2:	Test Substance Source	Low	TCEP was not analytically verified
	Metric 3:	Test Substance Purity	Medium	97% purity reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	the solvent concentration of treatments wasn't reported
	Metric 5:	Negative Control Response	High	good survival (>90%) in controls
	Metric 6:	Randomized Allocation	Low	did not report random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	adequate test system and description
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies reported between treatments
	Metric 9:	Measurement of Test Substance Concentration	High	test concentrations were measured
	Metric 10:	Exposure Duration and Frequency	High	adequate duration to observe desired effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate number of exposure groups to assess outcome
	Metric 12:	Testing at or Below Solubility Limit	High	well within solubility of TCEP
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately characterized
	Metric 14:	Acclimatization and Pretreatment Conditions	High	consistent pre treatment across all concentrations
	Metric 15:	Number of Organisms and Replicates per Group	Medium	four replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	test water parameters except temperature were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	adequate to determine percent hatch and survival
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Li, R., Wang, H., Mi, C., Feng, C., Zhang, L., Yang, L., Zhou, B. (2019). The adverse effect of TCIPP and TCEP on neurodevelopment of zebrafish embryos/larvae. Chemosphere 220811-817.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469290

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methodology was adequate for this outcome
	Metric 22:	Reporting of Data	High	data reporting was reasonably clear
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Movement assays for embryos (24 hpf) and larvae (120 hpf) were described in detail on page 7 of 19.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Embryo movement: "Specifically, overall patterns of activity within each cycle interval (ie, baseline, excitation, refractory) were compared with those in vehicle controls by (1) estimating the 50% peak difference from controls in either direction and (2) performing a Kolmogorov"Smirnov test that compared the empirical cumulative distribution function between chemical treatments and controls. A Bonferroni-corrected p-value threshold of .01 (0.05/5 treatments".01) was used to determine statistical significance."Larval Movement: "As larval activity did not meet parametric assumptions of normality, Kruskal"Wallis analyses of variance and Dunn"s multiple comparison post tests were used to compare median locomotor activity per minute in treatment versus controls in each of the 5-min light/dark phases."
	Metric 22:	Reporting of Data	High	All raw data across time for embryo and larval movement is reported in the supplemental notes (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for embryo movement assessment at 120 hpf for TCEP.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric	Rating	Comments
Overall Quality Determination		**High**	

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Mortality assessment was described in the section titled "Developmental malformation evaluations" but authors did not report specific criteria for death (ie, movement, heart-beat, color, etc.).
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"Data collection was undertaken using a custom barcoding and tracking system (Zebrafish Acquisition and Analysis Program) to facilitate reliable management of the large amounts of data collected. Statistical analyses were performed using R code with testing methodologies used by Truong et al. (2014) to evaluate developmental toxicity of chemicals under the ToxCast program(RCoreTeam, 2014; Truong et al., 2014). Briefly, a binomial testwas performed that calculated lowest effect levels (LELs) foreach endpoint to identify incidences that exceeded a significantthreshold above controls. This test was preferable to a logisticregression as it accounted for the observed nonmonotonicity offlame retardant toxicity."
	Metric 22:	Reporting of Data	High	All counts of data per treatment and control group are represented in the supplemental data for each compound and time point (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for Mortality assessment at 120 hpf for TCEP. The specific data and statistical significance for each compound are located within the supplemental PDF.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric	Rating	Comments
Overall Quality Determination		**High**	

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical is referred to by name, structure, and CASRN.
	Metric 2:	Test Substance Source	Low	Source was reported but the authors did not perform analytical verification.
	Metric 3:	Test Substance Purity	High	Purity is >98% and reported in Table 3.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (DMSO at 0.64%) was used.
	Metric 5:	Negative Control Response	High	All control responses are reported in the supplemental data PDF.
	Metric 6:	Randomized Allocation	Medium	Although not specifically reported as "random" the process of chorion removal and distribution into well plates was automated and is detailed in: "Mandrell, David, Lisa Truong, Caleb Jephson, Mushfiqur R. Sarker, Aaron Moore, Christopher Lang, Michael T. Simonich, and Robert L. Tanguay. "Automated zebrafish chorion removal and single embryo placement: optimizing throughput of zebrafish developmental toxicity screens." Journal of laboratory automation 17, no. 1 (2012): 66-74."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposures were static non-renewal from 6 to 120 hpf. Renewal of compounds would have been preferred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently among treatments and control.
	Metric 9:	Measurement of Test Substance Concentration	Low	No verification was performed, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Exposure was initiated at 6 hpf and continued till 120 hpf. The assessments at 24 and 120 hpf are appropriate to capture embryo and larval periods. 24 hour developmental assessments included: delays in developmental progression, notochord deformities, and altered spontaneous movements. 120 hour developmental assessments included: 17 developmental malformations, including yolk sac edema (YSE) and pericardial edema (PE); body axis (AXIS), trunk length (TRUN), caudal fin (CFIN), pectoral fin (PFIN), pigmentation (PIG), and somite (SOMI) deformities; eye (EYE), snout (SNOU), jaw (JAW), and otolith (OTIC) malformations; gross brain development (BRAIN); notochord (NC) and circulatory (CIRC) deformities; swim bladder presence and inflation (SWIM); and touch responses (TR).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations were: 64, 6.4, 0.64, 0.064, 0.0064, and 0 uM and represent a broad range of exposure concentrations.

Continued on next page . . .

. . . continued from previous page

Study Citation: Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome: Development/Growth
Chemical: Tris(2-chloroethyl) phosphate (TCEP)
HERO ID: 2953504

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (64 uM) is above the solubility limit. The remaining four concentrations are below solubility for TBBPA and TCEP. The highest two concentrations (6.4 and 64 uM) are above TPP solubility limit (solubility from final scopes for TBBPA, TPP, and TCEP are 4.1, 1.9 and 7.8 mg/L)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The strain and source for broodfish was reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Water quality parameters were well described and accordance with protocols under Oregon State University"s Institutional Animal Care and Use Committee.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	All sample size numbers for treatment and control groups are listed in the first two pages of the supplemental data PDF for this work.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Photoperiod and temperature were reported, while other water quality conditions were not.
	Metric 17:	Outcome Assessment Methodology	Medium	Developmental assessment was described in the section titled "Developmental malformation evaluations". The specifc analysis is detailed in another publication that authors referenced as HERO ID 8591199
	Metric 18:	Consistency of Outcome Assessment	High	Assessment was consistent among treatments and control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences in study groups based on environmental factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to indicate that differences were from animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Noyes, P. D., Haggard, D. E., Gonnerman, G. D., Tanguay, R. L. (2015). Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicological Sciences 145(1):177-195.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type (Tropical 5D); Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	2953504

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	"Data collection was undertaken using a custom barcoding and tracking system (Zebrafish Acquisition and Analysis Program) to facilitate reliable management of the large amounts of data collected. Statistical analyses were performed using R code with testing methodologies used by Truong et al. (2014) to evaluate developmental toxicity of chemicals under the ToxCast program(RCoreTeam, 2014; Truong et al., 2014). Briefly, a binomial testwas performed that calculated lowest effect levels (LELs) foreach endpoint to identify incidences that exceeded a significantthreshold above controls. This test was preferable to a logisticregression as it accounted for the observed nonmonotonicity offlame retardant toxicity."This reference is HERO ID 8591199
	Metric 22:	Reporting of Data	High	All counts of data per treatment and control group are represented in the supplemental data for each compound and time point (24 and 120 hpf).
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported by the authors.

Additional Comments: This form is for developmental assessment at 120 hpf for TCEP. The specific data and statistical significance for each compound are located within the supplemental PDF.

Overall Quality Determination High

Study Citation:	Sun, L., Xu, W., Peng, T.,ao, Chen, H., Ren, L.,in, Tan, H., Xiao, D.,an, Qian, H., Fu, Z. (2016). Developmental exposure of zebrafish larvae to organophosphate flame retardants causes neurotoxicity. Neurotoxicology and Teratology 5516-22.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469203

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP (CAS: 115-96-8; purity: 97%).
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	97% purity reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were used but solvent concentration in treatments was not reported.
	Metric 5:	Negative Control Response	Low	Survival response of controls not reported. "No treatment-related effects were found in the numbers of dead or malformed larvae for any chemical tested."
	Metric 6:	Randomized Allocation	Medium	"The embryos (b2 h post fertilization (hpf)) were randomly transferred into individual wells of 96-well plates (Corning, NY, USA) containing 100 "L of chemical solution."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test vessels were not covered.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations were not measured, but no reason to believe actual concentrations dissimilar from nominal.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number and spacing of groups was sufficient.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below solubility values.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"The zebrafish (Danio rerio) originated from the Institute of Hydrobiology of the Chinese Academy of Science (Wuhan, China). The fish maintenances were conducted according to the method of Westerfield (2000) with minor modification (Sun et al., 2010)"
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Control and treatment organisms treated similarly.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	"Twenty embryos in one plate were used for one replicate, with triplicate plates for each treatment."

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Xu, W., Peng, T.,ao, Chen, H., Ren, L.,in, Tan, H., Xiao, D.,an, Qian, H., Fu, Z. (2016). Developmental exposure of zebrafish larvae to organophosphate flame retardants causes neurotoxicity. Neurotoxicology and Teratology 5516-22.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469203

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	No details of test conditions were reported, but followed procedures from published peer-reviewed studies. "The fishmaintenances were conducted according to the method of Westerfield (2000) with minor modification (Sun et al., 2010)."
	Metric 17:	Outcome Assessment Methodology	High	Methods were reported for the outcomes of interest. "...the locomotor activity was measured by the Zebralab Video-track system..."
	Metric 18:	Consistency of Outcome Assessment	High	No inconsistencies were noted.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions and attrition were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among groups
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods clearly described. "Data were checked for normality and homogeneity of variance before conducting statistical comparison. As the assumptions were met,the data were subjected to one-way analysis of variance (ANOVA)followed by Dunnett's post hoc test"
	Metric 22:	Reporting of Data	High	Data was reported in a reasonably clear manner.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported

Additional Comments: This evaluation is for the behavioral outcome measured in zebrafish larvae following exposure to TCEP.

Overall Quality Determination High

Study Citation:	Sun, L., Xu, W., Peng, T.,ao, Chen, H., Ren, L.,in, Tan, H., Xiao, D.,an, Qian, H., Fu, Z. (2016). Developmental exposure of zebrafish larvae to organophosphate flame retardants causes neurotoxicity. Neurotoxicology and Teratology 5516-22.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Neurotoxicology
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469203

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP (CAS: 115-96-8; purity: 97%).
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich but the test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	97% purity reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were used but solvent concentration in treatments was not reported.
	Metric 5:	Negative Control Response	Low	Survival response of controls not reported. "No treatment-related effects were found in the numbers of dead or malformed larvae for any chemical tested."
	Metric 6:	Randomized Allocation	Medium	"The embryos (b2 h post fertilization (hpf)) were randomly transferred into individual wells of 96-well plates (Corning, NY, USA) containing 100 "L of chemical solution."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test vessels were not covered.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations were not measured, but no reason to believe actual concentrations dissimilar from nominal.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency were appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number and spacing of groups was sufficient.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below solubility values.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"The zebrafish (Danio rerio) originated from the Institute of Hydrobiology of the Chinese Academy of Science (Wuhan, China). The fish maintenances were conducted according to the method of Westerfield (2000) with minor modification (Sun et al., 2010)"
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Control and treatment organisms treated similarly.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	"Twenty embryos in one plate were used for one replicate, with triplicate plates for each treatment."
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Xu, W., Peng, T.,ao, Chen, H., Ren, L.,in, Tan, H., Xiao, D.,an, Qian, H., Fu, Z. (2016). Developmental exposure of zebrafish larvae to organophosphate flame retardants causes neurotoxicity. Neurotoxicology and Teratology 5516-22.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Neurotoxicology
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469203

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	No details of test conditions were reported, but followed procedures from published peer-reviewed studies. "The fishmaintenances were conducted according to the method of Westerfield (2000) with minor modification (Sun et al., 2010)."
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies for AChE activity measurements and gene transcription were provided in detail.
	Metric 18:	Consistency of Outcome Assessment	High	No inconsistencies were noted.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions and attrition were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among groups
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods clearly described. "Data were checked for normality and homogeneity of variance before conducting statistical comparison. As the assumptions were met,the data were subjected to one-way analysis of variance (ANOVA)followed by Dunnett's post hoc test"
	Metric 22:	Reporting of Data	High	Data was reported in a reasonably clear manner.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported

Additional Comments: This evaluation form is for the mechanistic data (AChE activity and gene transcription) in the reference.

Overall Quality Determination High

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA).
	Metric 2:	Test Substance Source	Low	percent purity was not analytically verified
	Metric 3:	Test Substance Purity	High	reported as 100% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	vehicle control reported with 0.1% DMSO
	Metric 5:	Negative Control Response	High	control mortality was <= 8.3%
	Metric 6:	Randomized Allocation	Medium	embryos were randomly distributed
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	documentation was adequate for this test system
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Medium	concentrations were measured but results were reported as log concentrations. "Based on the preliminary test, a gradient of nominal concentrationswas chosen (2.85, 28.5, 285, 14,250 and 28,500 mg/L, equalto 0.01, 0.1, 1, 50 and 100 mM, respectively)."
	Metric 10:	Exposure Duration and Frequency	High	3 to 120-h post fertilization
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	a wide range of concentrations was reported
	Metric 12:	Testing at or Below Solubility Limit	High	the use of DMSO should be adequate to ensure the highest concentrations were fully dissolved
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	age and source of organisms seemed satisfactory, although genus species was not specified by study authorsage and source of organisms seemed satisfactory"Adult zebrafish (4 months old, AB wild-type) were obtainedfrom the Institute of Hydrobiology, Chinese Academy of Sciences"
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	pretreatment was not well documented but it was adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	three replicates were used

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	minimal documentation of exposure water conditions
	Metric 17:	Outcome Assessment Methodology	Low	malformations were not well quantified
	Metric 18:	Consistency of Outcome Assessment	High	observations were made at multiple durations for all concentrations
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	malformations were not statistically analyzed
	Metric 22:	Reporting of Data	Low	data was reported in the form of typical images and in text but not quantified
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA).
	Metric 2:	Test Substance Source	Low	percent purity was not analytically verified
	Metric 3:	Test Substance Purity	High	reported as 100% purity"TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA)."
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	vehicle control reported with 0.1% DMSO
	Metric 5:	Negative Control Response	High	control mortality was <= 8.3%
	Metric 6:	Randomized Allocation	Medium	embryos were randomly distributed
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	documentation was adequate for this test system
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Medium	concentrations were measured but results were reported as log concentrations
	Metric 10:	Exposure Duration and Frequency	High	3 to 120-h post fertilization
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	a wide range of concentrations was reported"Expression of genes associated with estrogen receptor in zebrafish larvae (120hpf) exposure to 2.85, 28.5 and 285 mg TCEP/ L."
	Metric 12:	Testing at or Below Solubility Limit	High	the use of DMSO should be adequate to ensure the highest concentrations were fully dissolved
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	age and source of organisms seemed satisfactory
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	pretreatment was not well documented but it was adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	three replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	minimal documentation of exposure water conditions

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	assessment of endocrine gene expression was adequateThere were some concerns, however, regarding the different responses among exposure concentrations. Three concentrations of TCEP/L were asssessed, 2.85, 28.5, and 285 ug TCEP/L. Results for the vtg2, pgr, ncoal, ncoa3, er2b, vtg1, and er1 indicated a greater response for 2.85 and 285 ug TCEP/L than the middle 28.5 concentration. The authors did not provide a rationale as to why this may have occurred.
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"Key receptor pathway analysis was conducted according to a previous study (Liu et al., 2015). The resulting network genes (nodes) were colored by the Enhanced Graphics application within Cytoscape v3.1.1 (Cytoscape consortium, San Diego, CA, USA) according to the significant changes in gene expression in the respective treatments."
	Metric 22:	Reporting of Data	High	data was clearly reported graphically and in text
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: Due higher responses seen at the highest 285 ug TCEP/L and lowest 2.85 ug TCEP/L concentrations compared to the middle 28.5 ug TCEP/L concentrations, it is not clear which concentration is responsible for the effect seen.

Overall Quality Determination High

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Endocrine
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA).
	Metric 2:	Test Substance Source	Low	percent purity was not analytically verified
	Metric 3:	Test Substance Purity	High	reported as 100% purity"TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA)."
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	vehicle control reported with 0.1% DMSO
	Metric 5:	Negative Control Response	High	control mortality was <= 8.3%
	Metric 6:	Randomized Allocation	Medium	embryos were randomly distributed
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	documentation was adequate for this test system
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Medium	concentrations were measured but results were reported as log concentrations
	Metric 10:	Exposure Duration and Frequency	High	3 to 120-h post fertilization
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	a wide range of concentrations was reported"Expression of genes associated with estrogen receptor in zebrafish larvae (120hpf) exposure to 2.85, 28.5 and 285 mg TCEP/ L."
	Metric 12:	Testing at or Below Solubility Limit	High	the use of DMSO should be adequate to ensure the highest concentrations were fully dissolved
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	age and source of organisms seemed satisfactory
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	pretreatment was not well documented but it was adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	three replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	minimal documentation of exposure water conditions

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Endocrine
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	assessment of endocrine gene expression was adequateThere were some concerns, however, regarding the different responses among exposure concentrations. Three concentrations of TCEP/L were asssessed, 2.85, 28.5, and 285 ug TCEP/L. Results for the vtg2, pgr, ncoal, ncoa3, er2b, vtg1, and er1 indicated a greater response for 2.85 and 285 ug TCEP/L than the middle 28.5 concentration. The authors did not provide a rationale as to why this may have occurred.
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"Key receptor pathway analysis was conducted according to a previous study (Liu et al., 2015). The resulting network genes (nodes) were colored by the Enhanced Graphics application within Cytoscape v3.1.1 (Cytoscape consortium, San Diego, CA, USA) according to the significant changes in gene expression in the respective treatments."
	Metric 22:	Reporting of Data	High	data was clearly reported graphically and in text
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: Due higher responses seen at the highest 285 ug TCEP/L and lowest 2.85 ug TCEP/L concentrations compared to the middle 28.5 ug TCEP/L concentrations, it is not clear which concentration is responsible for the effect seen.

Overall Quality Determination High

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA).
	Metric 2:	Test Substance Source	Low	percent purity was not analytically verified
	Metric 3:	Test Substance Purity	High	reported as 100% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	vehicle control reported with 0.1% DMSO
	Metric 5:	Negative Control Response	High	control mortality was <= 8.3% "The morality rate of each concentration (2.85, 28.5and 28,500 mg TCEP/L) did not change rapidly (the change ofmortality rate was no more than 12.5%) from 24 to 120 hpf"
	Metric 6:	Randomized Allocation	Medium	embryos were randomly distributed
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	documentation was adequate for this test system
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Medium	concentrations were measured but results were reported as log concentrations
	Metric 10:	Exposure Duration and Frequency	High	3 to 120-h post fertilization
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	a wide range of concentrations was reported
	Metric 12:	Testing at or Below Solubility Limit	High	the use of DMSO should be adequate to ensure the highest concentrations were fully dissolved
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	age and source of organisms seemed satisfactory"Adult zebrafish (4 months old, AB wild-type) were obtainedfrom the Institute of Hydrobiology, Chinese Academy of Sciences"
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	pretreatment was not well documented but it was adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	three replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	minimal documentation of exposure water conditions

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	both percent mortality and log LC50 values were reported at multiple durations
	Metric 18:	Consistency of Outcome Assessment	High	observations were made at multiple durations for all concentrations
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	both percent mortality and Log LC50 values reported at various durations
	Metric 22:	Reporting of Data	High	data was reported graphically and in text
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP was purchased from AccuStandard (100% , AccuStandardInc., CT, USA).
	Metric 2:	Test Substance Source	Low	percent purity was not analytically verified
	Metric 3:	Test Substance Purity	High	reported as 100% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	vehicle control reported with 0.1% DMSO
	Metric 5:	Negative Control Response	Low	control response was not reported
	Metric 6:	Randomized Allocation	Medium	embryos were randomly distributed
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	documentation was adequate for this test system
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted by the study authors
	Metric 9:	Measurement of Test Substance Concentration	High	concentrations were measured but actual values were not reported except as the Log value of the mean-measured concentrations
	Metric 10:	Exposure Duration and Frequency	High	3 to 120-h post fertilization
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	a wide range of concentrations was reported
	Metric 12:	Testing at or Below Solubility Limit	High	the use of DMSO should be adequate to ensure the highest concentrations were fully dissolved
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	age and source of organisms seemed satisfactory, although genus species was not specified by study authorsage and source of organisms seemed satisfactory"Adult zebrafish (4 months old, AB wild-type) were obtainedfrom the Institute of Hydrobiology, Chinese Academy of Sciences"
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	pretreatment was not well documented but it was adequate
	Metric 15:	Number of Organisms and Replicates per Group	Medium	number of organisms and replicates (3) were adequate
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Wu, Y., Su, G., Tang, S., Liu, W., Ma, Z., Zheng, X., Liu, H., Yu, H. (2017). The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish. Chemosphere 168:122-130.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; AB strain; Embryo
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469243

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	minimal documentation of exposure water conditions"Moreover, the morality rate of each concentration (2.85, 28.5and 28,500 mg TCEP/L) did not change rapidly (the change ofmortality rate was no more than 12.5%) from 24 to 120 hpf (seeSupplementary Table S2), indicating the amount of chemicalentering the zebrafish did not change greatly with increasing ofexposure concentrations, consistent with the relatively low log Kow(1.44)." The second half of that statement seems to indicate that, contrary to what the study authors have concluded (a relationship between bioaccumulation and TCEP exposure), increasing the TCEP concentrations had no effect on bioaccumulation by zebrafish.
	Metric 17:	Outcome Assessment Methodology	Medium	The bioaccumulation potency of TCEP was calculated based on the ratios of concentrations between zebrafish larvae and exposure solutions.
	Metric 18:	Consistency of Outcome Assessment	Medium	control measurements were not well documented, other sources were cited and were reviewed to determine assessment methods
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unexpected outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	a linear regression equation was established and there was little statistical difference between nominal and measured test concentrations
	Metric 22:	Reporting of Data	Medium	actual measured test concentrations that accumulation was based on, was only available in log values
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported, expected outcomes had explanations provided

Additional Comments: None

Overall Quality Determination High

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as tris (2-chloroethyl) phosphate (TCEP; CAS no. 115-96-8). Chemical structure was given in table 1.
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group containing 0.01% DMSO was included.
	Metric 5:	Negative Control Response	High	Control group response for behavior assay were reported in the text and in figures 1 and 2.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups. Non-deformed larvae were selected for behavior assay
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system, test solution renewed every 24 hrs.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among all test groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration of 96 hours was appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study. 4 concentrations and a control (5 mg/L, 25 mg/L, 125 mg/L, 625 mg/L) w were used.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the solubility limit. The solvent concentration was appropriate.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Japanese medaka (d-rR strain) originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 test organisms with 3 replicates per treatment.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions were adequate during incubation period. However, environmental conditions were not reported for test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology (locomotor behavior assay) was appropriate and reported the intended outcomes of interest. End points were changes in the relative swimming speed during 30 min of visible light and during the dark-light-dark photoperiod stimulation test
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions during test were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Appropriate statistics were used after data met assumptions of the tests.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings (changes in the relative swimming speed during 30 min of visible light and during the dark-light-dark photoperiod stimulation test; figures 1 and 2) were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation form is relevant to the behavioral outcome (changes in the relative swimming speed during 30 min of visible light and during the dark-light-dark photoperiod stimulation test) in medaka larvae following exposure to TCEP.

Overall Quality Determination High

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as tris (2-chloroethyl) phosphate (TCEP; CAS no. 115-96-8). Chemical structure was given in table 1.
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group containing 0.01% DMSO was included.
	Metric 5:	Negative Control Response	High	Control group response for AChE and mRNA expression were reported in the text and in Figures 3 and 4. Control responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system, test solution renewed every 24 hrs.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among all test groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (96 hours) appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study. 4 concentrations and a control (5 mg/L, 25 mg/L, 125 mg/L, and 625 mg/L).
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the solubility limit. The solvent concentration was appropriate.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Japanese medaka (d-rR strain) originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	For gene transcription analysis, 15 larvae from each replicate (from the behavior assay) were pooled into 1 sample. For AChE activity measurements, 30 larvae/beaker were sampled and homogenized after the exposure period, and 3 replicates of each concentration were used.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions were adequate during incubation period. However, environmental conditions were not reported for test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies (for acetylcholinesterase activity and gene transcription analysis) was appropriate and reported the intended outcomes of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions during test were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Appropriate statistics were used after data met assumptions of the tests.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings for AChE activity and mRNA level (Figures 3 and 4) were presented for each treatment and control group and were adequate to determine values for the endpoints of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation form is relevant to the mechanistic endpoints (AChE activity and changes in the transcription of genes related to the nervous system) in medaka larvae following exposure to TPP.

Overall Quality Determination High

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as tris (2-chloroethyl) phosphate (TCEP; CAS no. 115-96-8). Chemical structure was given in table 1.
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group containing 0.01% DMSO was included.
	Metric 5:	Negative Control Response	High	Control group response (table 2) was adequate.
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated for treatments.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system, test solution renewed every 24 hrs.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among all test groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (14 days) appropriate for test. "During the exposure period, embryos went through the major embryonic stages (blastula, gastrula, neurula, and organogenesis), hatching and proceeding into the larval stage."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and dose spacing were appropriate for test. 4 concentrations and a control (5 mg/L, 25 mg/L, 125 mg/L, and 625 mg/L). Concentrations were determined via a prior range finding test (data not shown).
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the solubility limit. The solvent concentration was appropriate.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Japanese medaka (d-rR strain) originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 test organisms with 3 replicates per treatment.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Cardiovascular
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions were adequate during incubation period. However, environmental conditions were not reported for test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Assessment methods for body length, hatch % , incubation time, deformity % and heart rate were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Appropriate statistics were used after data met assumptions of the tests.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for hatchability, time to hatching, gross abnormality rate, heart rate and body length.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation form is relevant to cardiovascular outcome (heart rate) determined in medaka embryos following exposure to TCEP.

Overall Quality Determination High

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as tris (2-chloroethyl) phosphate (TCEP; CAS no. 115-96-8). Chemical structure was given in table 1.
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group containing 0.01% DMSO was included.
	Metric 5:	Negative Control Response	High	Control group response (table 2) was adequate.
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated for treatments.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system, test solution renewed every 24 hrs.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among all test groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (14 days) appropriate for test. "During the exposure period, embryos went through the major embryonic stages (blastula, gastrula, neurula, and organogenesis), hatching and proceeding into the larval stage."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study. 4 concentrations and a control (5 mg/L, 25 mg/L, 125 mg/L, and 625 mg/L). Concentrations were determined via a prior range finding test (data not shown).
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the solubility limit. The solvent concentration was appropriate.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Japanese medaka (d-rR strain) originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 test organisms with 3 replicates per treatment.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions were adequate during incubation period. However, environmental conditions were not reported for test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methods for body length, hatch % , incubation time, deformity % and heart rate were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions during test were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Appropriate statistics were used after data met assumptions of the tests.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for hatchability, time to hatching, gross abnormality rate, heart rate and body length.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation form is relevant to reproductive outcomes (percent hatchability and time to hatch) determined in medaka embryos following exposure to TCEP.

Overall Quality Determination High

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as tris (2-chloroethyl) phosphate (TCEP; CAS no. 115-96-8). Chemical structure was given in table 1.
	Metric 2:	Test Substance Source	Low	Purchased from Sigma-Aldrich. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group containing 0.01% DMSO was included.
	Metric 5:	Negative Control Response	High	Control group response (table 2) was adequate.
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated for treatments.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system, test solution renewed every 24 hrs.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among all test groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (14 days) appropriate for test. "During the exposure period, embryos went through the major embryonic stages (blastula, gastrula, neurula, and organogenesis), hatching and proceeding into the larval stage."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and dose spacing were appropriate for test. 4 concentrations and a control (5 mg/L, 25 mg/L, 125 mg/L, and 625 mg/L). Concentrations were determined via a prior range finding test (data not shown).
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the solubility limit. The solvent concentration was appropriate.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Japanese medaka (d-rR strain) originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 test organisms with 3 replicates per treatment.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Sun, L., Tan, H., Peng, T., Wang, S., Xu, W., Qian, H., Jin, Y., Fu, Z. (2016). Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 35(12):2931-2940.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oryzias latipes*; d-rR strain; Embryo
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	4292102

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions were adequate during incubation period. However, environmental conditions were not reported for test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Assessment methods for body length, hatch % , incubation time, deformity % and heart rate were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Appropriate statistics were used after data met assumptions of the tests.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for hatchability, time to hatching, gross abnormality rate, heart rate and body length.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation form is relevant to development/growth outcomes (body length and percentage of gross abnormality rate) determined in medaka embryos following exposure to TCEP.

Overall Quality Determination High

Study Citation:	Life Sciences Research Ltd, (1990). Fyrol CEF: Acute toxicity to rainbow trout.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Juvenile
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	6310866

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name, CASNR, and structure provided
	Metric 2:	Test Substance Source	High	Supplied by AKZO Chemical Inc., Batch no. 8101 K-1-4
	Metric 3:	Test Substance Purity	Low	Contrary to narrative, purity was not reported in appendix 2.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used.
	Metric 5:	Negative Control Response	High	Zero mortality reported for controls
	Metric 6:	Randomized Allocation	Medium	Random allocation was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Only nominal concentrations used. Measures taken to account for volatility (e.g., head space, closed system etc.) not reported.
	Metric 8:	Consistency of Exposure Administration	High	exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were used.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing appropriate for the test.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below water solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Fish and source were well described with the exception of sex.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation was appropriate for test.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent across groups.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Life Sciences Research Ltd, (1990). Fyrol CEF: Acute toxicity to rainbow trout.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Juvenile
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	6310866

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No differences among groups reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analyses were performed. However, statistics used were reported as not valid for the data. Therefore approximate values were reported.
	Metric 22:	Reporting of Data	High	Data reported for all outcomes.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Life Sciences Research Ltd, (1990). Fyrol CEF: Acute toxicity to rainbow trout.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Juvenile
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	6310866

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name, CASNR, and structure provided
	Metric 2:	Test Substance Source	High	Supplied by AKZO Chemical Inc., Batch no. 8101 K-1-4
	Metric 3:	Test Substance Purity	Low	Contrary to narrative, purity was not reported in appendix 2.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used.
	Metric 5:	Negative Control Response	Low	No data on control conditions related to the outcome for this form (loss of coordination, pigmentation, and edema) are reported or quantified.
	Metric 6:	Randomized Allocation	Medium	Random allocation was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Only nominal concentrations used. Measures taken to account for volatility (e.g., head space, closed system etc.) not reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were used.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing appropriate for the test.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below water solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Fish and source were well described with the exception of sex.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation was appropriate for test.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	Low	This form is for observations reported in the results on loss of equilibrium, pigmentation, and edema (Page 18/24). This report has no data sheets to quantify these observations past report in the results. Responses for control individuals are not reported.
	Metric 18:	Consistency of Outcome Assessment	Low	The methods do not report the frequency of when this outcome was recorded.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Life Sciences Research Ltd, (1990). Fyrol CEF: Acute toxicity to rainbow trout.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Juvenile
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	6310866

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No differences among groups reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	No Statistics for these observations were reported.
	Metric 22:	Reporting of Data	Low	The data for the observations for this form are not presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: This form is for observations reported in the results on loss of equilibrium, pigmentation, and edema (Page 18/24). This report has no data sheets to quantify these observations past report in the results. Responses for control individuals are not reported.

Overall Quality Determination Medium

Study Citation:	Arukwe, A., Carteny, C. C., Eggen, T. (2016). Lipid peroxidation and oxidative stress responses in juvenile salmon exposed to waterborne levels of the organophosphate compounds tris(2-butoxyethyl)- and tris(2-chloroethyl) phosphates. Journal of Toxicology and Environmental Health, Part A: Current Issues 79(13-15):515-525.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo salar*; Juvenile
Health Outcome:	Mechanistic-Cell signaling/function-Oxidative stress (including redox biology)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469341

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Th tests substance was identified by chemical name and molecular formula.
	Metric 2:	Test Substance Source	Low	The test substance was obtained from obtained from Sigma-Aldrich Chemie GmbH but its was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity of TCEP was reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were reported. A semistatic experimental protocol was used. Test solution were prepared in Milli-Q water. Test solutions were renewed every 3 days.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	7-d exposure duration is appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Three exposure groups (0.04, 0.2, or 1 mg/L) and a control were used and adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described. Average length and weight measurements were provided. Test organisms were obtained from Settefiskanlegget Lundamo AS (Lundamo, Norway).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for 9 days and all pretreatment conditions were the same for control and exposed organisms
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers of test organisms was reported. "Fish were divided into 6 treatments and 1 control where each group constitutes 16 individuals." The use of replicates was not reported.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Arukwe, A., Carteny, C. C., Eggen, T. (2016). Lipid peroxidation and oxidative stress responses in juvenile salmon exposed to waterborne levels of the organophosphate compounds tris(2-butoxyethyl)- and tris(2-chloroethyl) phosphates. Journal of Toxicology and Environmental Health, Part A: Current Issues 79(13-15):515-525.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo salar*; Juvenile
Health Outcome:	Mechanistic-Cell signaling/function-Oxidative stress (including redox biology)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469341

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions at which the tanks were maintained were appropriate. "The tanks were kept at a constant temperature of 8°C and a 12:12-h photoperiod." However, water quality parameters were (pH, DO, etc.) were not monitored.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. Methodologies to determine gene expression of GPx, GR, GST, peroxisome proliferator-activated receptors (PPAR) were described well. The immunochemical method to determine presence of PPAR proteins was also described well.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described. "One-way analysis of variance (ANOVA) was performed, followed by post hoc analysis (Duncan"s test) between subgroups."
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group for gene expression data. The immunoblot analysis results (Figure 1D) is missing in the manuscript.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: This form includes all mechanistic data- gene expression of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), peroxisome proliferator-activated receptors (PPAR), and presence of PPAR proteins using immunochemical methods.

Overall Quality Determination High

Study Citation:	Kovacevic, V., Simpson, A. J., Simpson, M. J. (2018). Investigation of daphnia magna sub-lethal exposure to organophosphate esters in the presence of dissolved organic matter using "H NMR-based metabolomics. Metabolites 8(2):34.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; *Daphnia magna*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5184752

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and chemical structure (Table 1).
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 97% .
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group, both DOM and clean controls were used.
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups was reported for analyzed metabolites but not for survival.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. Stock solution was prepared in dechlorinated water and beakers with test solution "were sealed with parafilm and equilibrated on a magnetic stirrer at room temperature in the dark for 48 h."
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods, and were similar to nominals.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported (48 hours) and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentration was below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. Laboratory cultured adult daphnids (16 days old) were used.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported, 10 replicates with 10 daphnids each.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Kovacevic, V., Simpson, A. J., Simpson, M. J. (2018). Investigation of daphnia magna sub-lethal exposure to organophosphate esters in the presence of dissolved organic matter using "H NMR-based metabolomics. Metabolites 8(2):34.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; *Daphnia magna*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Nutritional and Metabolic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5184752

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Daphnia culture conditions were reported. Organism housing and feeding during exposure period were reported. Biomass loading (1 daphnid/30 ml) was appropriate. It was stated that temperature and light conditions were the same as the culture conditions but specifics during the exposure period were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest. Metabolomics methods were described well.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups in environmental conditions although specifics were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described. "The statistical significance of the individual metabolite percent changes was determined with a t-test (two-tailed, equal variances, $p < 0.05$) and the results of this statistical analysis is given in Table S3 in the Supplementary Materials."
	Metric 22:	Reporting of Data	High	Table S3 has statistical results for all treatments.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained

Additional Comments: The study examined the effect of dissolved organic matter (DOM) on the sub-lethal toxicity of TCEP to D. magna using 1H NMR-based metabolomics. TCEP was tested alone and in the presence of DOM.

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN in supplemental notes.
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable and presented within the supplemental excel file.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	High	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported in supplemental excel file.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	High	Data reported in supplemental excel file.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: supplementary data (table 2) provides LC50 values for 12 day worms but TCEP is not listed in this data. Specific data on mortality for TCEP is within the excel file (no mortality for worms at concentrations in this work).

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	Low	Data were reported in supplemental, however reporting of data was not clear.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This form represents measures of growth, specifically eye regeneration on day 7. Data is located in the supplementary excel file.

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	High	Data were reported in supplemental excel file.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This form is for the behavior metrics which on day 7 are unstimulated behavior and phototaxsis. The TCEP data for this metric is located in the supplementary Excel file.

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported in supplemental excel file.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	High	Data reported in supplemental excel file.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: supplementary data (table 2) provides LC50 values for 12 day worms but TCEP is not listed in this data. Specific data on mortality for TCEP is within the excel file (no mortality for worms at concentrations in this work).

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	Low	Data were reported in supplemental, however reporting of data was not clear.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This form identifies morphology assessments at day 12 of exposure. This is identified in Figure one of the paper. Results are located on page 9/18, TCEP was not detailed for these data.

Overall Quality Determination High

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by name and CASRN
	Metric 2:	Test Substance Source	Low	Source of stock solutions were identified. Analytical verification not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent controls were reported.
	Metric 5:	Negative Control Response	High	Negative control response acceptable.
	Metric 6:	Randomized Allocation	Low	Worms were selected by specified size.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system well described.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Stock solutions of different concentrations were purchased. No analytical measurements were reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure concentration and frequency were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of exposure groups and spacing were appropriate for the tests.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration (100 uM) is 28 mg/L which is over the solubility listed in the final scope (7.9 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation and pretreatments were appropriate for tests.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms and replicates were acceptable.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome methodology was consistent.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Hagstrom, D., Hayes, P., Graham, A., Collins, E. S. (2019). Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167(1):26-44.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	10064285

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics well described.
	Metric 22:	Reporting of Data	High	Data were reported in supplemental excel file.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This form represents behavior metrics in the paper that include: unstimulated behavior, thermotaxsis, phototaxis, and scrunching. These data for TCEP are located in the supplementary excel file.

Overall Quality Determination High

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. "Negative" controls contained either acetaminophen or L-ascorbic acid. A positive solvent control (0.5% DMSO) was also used. The authors presented information from previous studies to verify that acetaminophen or L-ascorbic acid did not affect the test organisms.
	Metric 5:	Negative Control Response	High	100% survival in solvent control (0.5% DMSO), no mortality observed in "negative" controls
	Metric 6:	Randomized Allocation	Low	No mention of randomization in this paper. Author did cite another paper which may have described randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail and appropriately accounted for the physical-chemical properties of the test substance (solvent used due to the low solubility of the test material). Measured test concentrations were not provided.
	Metric 8:	Consistency of Exposure Administration	High	No inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, highest concentration listed as 99.5 uM in supplemental data instead of a nominal value of 100 uM as stated in text
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to cause mortality for some of the chemicals in this study (but not TCEP).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations seemed adequate to illicit a response (mortality) for most chemicals tested in this study. This was below the NOAEL for TCEP, but the purpose of the study was to compare the mortality between chemicals within a specific group, so this was not determined to affect the rating of this study.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional mortality in some chemicals in the screen (No mortality was observed for TCEP, so this was not an issue for this chemcial).
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment was adequate to describe the comparative mortality of several flame retardants following exposure to the test material.
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	Low	The authors describe the statistical procedure as: "Statistical significance was determined using either a one-tailed Fisher's exact test for lethality, eye regeneration, phototaxis, and scrunching endpoints; Mann Whitney U test for thermotaxis and unstimulated behavior; or two-tailed t-test for unstimulated behavior (depending on normality of the sample) using a significance level of 0.05. This was adequate to determine if mortality was significant." This analysis and the raw data describing mortality were not presented in this study.
	Metric 22:	Reporting of Data	Low	A large amount of data was presented in the supplemental file (Zhang et al., 2019): https://doi.org/10.1016/j.ntt.2019.03.003. Data were not clear and interpretation was difficult. It appeared that no mortality was observed in the adults following exposure to TCEP for 7-12 days, but this was not clearly discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	no mortalities for entire test duration in any treatment. The lack of bioactivity observed in this study for TCEP was not consistent with previous studies showing bioactivity in Zebrafish. This was explained in the discussion (4.6)

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. "Negative" controls contained either acetaminophen or L-ascorbic acid. A positive solvent control (0.5% DMSO) was also used. The authors presented information from previous studies to verify that acetaminophen or L-ascorbic acid did not affect the test organisms.
	Metric 5:	Negative Control Response	High	100% survival in solvent control (0.5% DMSO), no mortality observed in "negative" controls.
	Metric 6:	Randomized Allocation	Low	no mention of randomization in this paper. Author did cite another paper which may have described randomization
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	minimal details provided making acceptability difficult to determine
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	exposure concentrations were not measured, high concentration listed as 99.5 uM in supplemental data instead of a nominal value of 100 uM as stated in text
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to cause mortality for some of the chemicals in this study (but not TCEP).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations seemed adequate to illicit a response (mortality) for most chemicals tested in this study. This was below the NOAEL for TCEP, but the purpose of the study was to compare the mortality between chemicals within a specific group, so this was not determined to affect the rating of this study.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional mortality in some chemicals in the screen (No mortality was observed for TCEP, so this was not an issue for this chemcial).
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment was adequate to describe the comparative mortality of several flame retardants following exposure to the test material.
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	Low	The authors describe the statistical procedure as: "Statistical significance was determined using either a one-tailed Fisher's exact test for lethality, eye regeneration, phototaxis, and scrunching endpoints; Mann Whitney U test for thermotaxis and unstimulated behavior; or two-tailed t-test for unstimulated behavior (depending on normality of the sample) using a significance level of 0.05. This was adequate to determine if mortality was significant." This analysis and the raw data describing mortality were not presented in this study.
	Metric 22:	Reporting of Data	Low	A large amount of data was presented in the supplemental file (Zhang et al., 2019): https://doi.org/10.1016/j.ntt.2019.03.003. Data were not clear and interpretation was difficult. It appeared that no mortality was observed in the regenerating adults following exposure to TCEP for 7-12 days, but this was not clearly discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	no mortalities for entire test duration in any treatment. The lack of bioactivity observed in this study for TCEP was not consistent with previous studies showing bioactivity in Zebrafish. This was explained in the discussion (4.6)

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. "Negative" controls contained either acetaminophen or L-ascorbic acid. A positive solvent control (0.5% DMSO) was also used. The authors presented information from previous studies to verify that acetaminophen or L-ascorbic acid did not affect the test organisms.
	Metric 5:	Negative Control Response	High	100% survival in solvent control (0.5% DMSO), no mortality observed in "negative" controls
	Metric 6:	Randomized Allocation	Low	no mention of randomization in this paper. Author did cite another paper which may have described randomization
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail and appropriately accounted for the physical-chemical properties of the test substance (solvent used due to the low solubility of the test material). Measured test concentrations were not provided.
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, highest concentration listed as 99.5 uM in supplemental data instead of a nominal value of 100 uM as stated in text
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to elicit a response for the chemicals in this study (but not TCEP).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations seemed adequate to illicit a response (development) for most chemicals tested in this study. This was below the NOAEL for TCEP, but the purpose of the study was to compare the mortality between chemicals within a specific group, so this was not determined to affect the rating of this study.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional mortality in some chemicals in the screen (No effects on development were observed for TCEP, so this was not an issue for this chemcial).
	Metric 17:	Outcome Assessment Methodology	High	outcome assessment was adequate, TCEP was essentially inactive for this test
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	High	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	Low	The authors describe the statistical procedure as: "Statistical significance was determined using either a one-tailed Fisher's exact test for lethality, eye regeneration, phototaxis, and scrunching endpoints; Mann Whitney U test for thermotaxis and unstimulated behavior; or two-tailed t-test for unstimulated behavior (depending on normality of the sample) using a significance level of 0.05. This was adequate to determine if mortality was significant." This analysis and the raw data describing mortality were not presented in this study.
	Metric 22:	Reporting of Data	Low	A large amount of data was presented in the supplemental file (Zhang et al., 2019): https://doi.org/10.1016/j.ntt.2019.03.003. Data were not clear and interpretation was difficult. It appeared that no effect on development were observed in the adults following exposure to TCEP for 7-12 days, but this was not clearly discussed.
	Metric 23:	Explanation of Unexpected Outcomes	High	No effects on development were observed for entire test duration in any treatment for TCEP. The lack of bioactivity observed in this study for TCEP was apparently not consistent with previous studies showing bioactivity in Zebrafish. This was explained in the discussion (4.6)

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. "Negative" controls contained either acetaminophen or L-ascorbic acid. A positive solvent control (0.5% DMSO) was also used. The authors presented information from previous studies to verify that acetaminophen or L-ascorbic acid did not affect the test organisms.
	Metric 5:	Negative Control Response	High	No effects observed in "negative" or solvent controls
	Metric 6:	Randomized Allocation	Low	No mention of randomization in this paper. Author did cite another paper which may have described randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail and appropriately accounted for the physical-chemical properties of the test substance (solvent used due to the low solubility of the test material). Measured test concentrations were not provided.
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	exposure concentrations were not measured, high concentration listed as 99.5 uM in supplemental data instead of a nominal value of 100 uM as stated in text
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to to illicit a response for some of the chemicals in this study (but not TCEP).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations seemed adequate to illicit a response (behavior) for most chemicals tested in this study. This was below the NOAEL for TCEP, but the purpose of the study was to compare the effects on behavior between chemicals within a specific group, so this was not determined to affect the rating of this study.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional mortality in some chemicals in the screen (No mortality was observed for TCEP, so this was not an issue for this Chemical).
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment was adequate to describe the comparative mortality of several flame retardants following exposure to the test material.
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; regenerative; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	Low	The authors describe the statistical procedure as: "Statistical significance was determined using either a one-tailed Fisher's exact test for lethality, eye regeneration, phototaxis, and scrunching endpoints; Mann Whitney U test for thermotaxis and unstimulated behavior; or two-tailed t-test for unstimulated behavior (depending on normality of the sample) using a significance level of 0.05. This was adequate to determine if mortality was significant." This analysis and the raw data describing behavioral effects were not presented in this study.
	Metric 22:	Reporting of Data	Low	A large amount of data was presented in the supplemental file (Zhang et al., 2019): https://doi.org/10.1016/j.ntt.2019.03.003. Data were not clear and interpretation was difficult. It appeared that no behavioral effects were observed in the regenerating worms following exposure to TCEP for 7-12 days, but this was not clearly discussed or adequate to characterize a dose response.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes for entire test duration in any treatment

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Other (please specify below) (AChE)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. A positive solvent control (0.5% DMSO) was used.
	Metric 5:	Negative Control Response	High	No effects on ACHE activity was reported in the solvent control
	Metric 6:	Randomized Allocation	Low	No mention of randomization in this paper. Author did cite another paper which may have described randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail and appropriately accounted for the physical-chemical properties of the test substance (solvent used due to the low solubility of the test material). Measured test concentrations were not provided.
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, as this was conducted as a limit test, only one test concentration with a nominal value of 100 uM was used.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to elicit significant loss of cholinesterase activity for the other chemical in this study, EHDP, but not TCEP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	As this was conducted as a limit test, only one concentration was used and no effects were observed.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Other (please specify below) (AChE)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Low	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional effects in the screen (No effects were observed following exposure to TCEP, so this was not an issue for this chemical).
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment was adequate to describe the comparative loss of cholinesterase activity following exposure to two flame retardants, TCEP and EHDP.
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	statistical analysis not applied as this was a limit test
	Metric 22:	Reporting of Data	Low	Data were not clear and interpretation was difficult. It appeared that no loss of cholinesterase activity in the adults following exposure to TCEP for 7-12 days, but this was not clearly discussed and raw data were not available.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Other (please specify below) (AChE)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 23:	Explanation of Unexpected Outcomes	High	No mortalities for entire test duration in any treatment. The lack of bioactivity observed in this study for TCEP was not consistent with previous studies showing bioactivity in Zebrafish. This was explained in the discussion (4.6) as being related to the loss of cholinesterase activity.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	clearly identified with name and CAS #
	Metric 2:	Test Substance Source	Low	Sourced from Sigma Aldrich, but chemical was not analytically verified by the laboratory.
	Metric 3:	Test Substance Purity	High	reported as 99.07% pure
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Authors did not use a true negative control. "Negative" controls contained either acetaminophen or L-ascorbic acid. A positive solvent control (0.5% DMSO) was also used. The authors presented information from previous studies to verify that acetaminophen or L-ascorbic acid did not affect the test organisms.
	Metric 5:	Negative Control Response	High	No effects observed in "negative" or solvent controls
	Metric 6:	Randomized Allocation	Low	no mention of randomization in this paper. Author did cite another paper which may have described randomization
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail and appropriately accounted for the physical-chemical properties of the test substance (solvent used due to the low solubility of the test material). Measured test concentrations were not provided.
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noticed or reported
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, high concentration listed as 99.5 uM in supplemental data instead of a nominal value of 100 uM as stated in text
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration (12 days) was adequate to to illicit a response for some of the chemicals in this study (but not TCEP, as no activity was observed up to the highest test concentration).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations seemed adequate to illicit a response (behavior) for most chemicals tested in this study. This was below the NOAEL for TCEP, but the purpose of the study was to compare the effects on behavior between chemicals within a specific group, so this was not determined to affect the rating of this study.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (0.5% DMSO) was used to facilitate the dissolution of the test material into the test medium.
Domain 4: Test Organism				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Supplemental methods description describes the test organism in more detail (Zhang et al., 2019). Test organisms were described as adults and "Test worms were manually selected to fall within a certain range of sizes and we found full worm length, after automated size measurement, to be 7.3 6 2.3 mm (mean 6 SD), and tail worm length to be 7.3 mm "/- 2.7 mm (mean 6 SD).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In supplemental materials, pretreatment procedure was described as: "Animals were starved for at least 5 days before beingused for experiments and their containers were cleaned immediately prior to worm selection for experiments." This was adequate for gut-clearance.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	8 individuals were used for each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Test conditions were fully described in supplemental methods paper (Zhang et al., 2019): "Wells of a 48- well plate with 200 ml of IO water." The authors noted that in the present study, they updated the feeding procedure, but this was not described clearly, "we fed planarians used in FR screen 2 commercial freeze-dried organic chicken liver (Amazon, Seattle, WA) to better control food quality and thus minimize animal fitness variability." It was unclear when this supplemental feeding occurred during the exposure period or if the lack of feeding could have contributed to additional mortality in some chemicals in the screen (No mortality was observed for TCEP, so this was not an issue for this Chemical).
	Metric 17:	Outcome Assessment Methodology	Medium	Outcome assessment was adequate to describe the comparative behavioral changes following exposure to the test material and other flame retardants.
	Metric 18:	Consistency of Outcome Assessment	High	assessment methods seemed consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Besides the feeding procedure, which did not contribute to mortality for TCEP, there were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Zhang, S., Ireland, D., Sipes, N. S., Behl, M., Collins, E. S. (2019). Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and Teratology 7354-66.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Dugesia japonica*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469417

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The authors describe the statistical procedure as: "Statistical significance was determined using either a one-tailed Fisher's exact test for lethality, eye regeneration, phototaxis, and scrunching endpoints; Mann Whitney U test for thermotaxis and unstimulated behavior; or two-tailed t-test for unstimulated behavior (depending on normality of the sample) using a significance level of 0.05. This was adequate to determine if mortality was significant." This analysis and the raw data describing behavioral effects were not presented in this study.
	Metric 22:	Reporting of Data	High	A large amount of data was presented in the supplemental file (Zhang et al., 2019): https://doi.org/10.1016/j.ntt.2019.03.003. Data were not clear and interpretation was difficult. It appeared that no behavioral effects were observed in the regenerating worms following exposure to TCEP for 7-12 days, but this was not clearly discussed or adequate to characterize a dose response.v
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes for entire test duration in any treatment

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	Hepatic/Liver
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	identified in text and abstract
	Metric 2:	Test Substance Source	Low	TCEP was purchased from Sigma-Aldrich but not analytically verified
	Metric 3:	Test Substance Purity	High	purity reported as > 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control of safflower oil (the delivery medium) was used.
	Metric 5:	Negative Control Response	High	No reported mortalities in the negative controls and significant differences in the effects were observed between the controls and exposure groups.
	Metric 6:	Randomized Allocation	Low	random dosing was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure preparation was well documented. Each kestrel ate one cockerel per day that had been injected with 50 "L of a safflower oil solution containing TCEP. Dosage was calculated by weight to ensure consistent weight between the flame retardants used int he study.
	Metric 8:	Consistency of Exposure Administration	High	No inconsistencies were reported
	Metric 9:	Measurement of Test Substance Concentration	High	actual measured concentrations were reported
	Metric 10:	Exposure Duration and Frequency	High	exposure duration (21 days) was long enough to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Authors used only one test concentration, normalized by body weight, across multiple chemicals so dosage spacing is not relevant in this case
	Metric 12:	Testing at or Below Solubility Limit	N/A	dietary dose was administered via vegetable oil carrier, therefore the solubility was not relevant
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately described and appropriate
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	no description of acclimatization process was provided
	Metric 15:	Number of Organisms and Replicates per Group	Low	7 individuals for each chemical with no replication. Given that the intention was to characterize the comparative effects of the test material and not to calculate a dose response, this is sufficient for statistical analysis

Continued on next page . . .

. . . continued from previous page

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	Hepatic/Liver
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	test conditions were adequate and reasonably well documented
	Metric 17:	Outcome Assessment Methodology	High	methods were adequate to assess hepatic and thyroid hormone levels
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	End points were compared for each treatment to vehicle-treated controls by parametric analysis of variance (ANOVA). Calculations were not provided
	Metric 22:	Reporting of Data	High	data was reported adequately and clearly
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	Other (please specify below) (Thyroid Function)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	identified in text and abstract
	Metric 2:	Test Substance Source	Low	TCEP was purchased from Sigma-Aldrich but not analytically verified
	Metric 3:	Test Substance Purity	High	purity reported as > 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative control of safflower oil (the delivery medium) was used
	Metric 5:	Negative Control Response	High	No reported mortalities in the negative controls and significant differences in the effects were observed between the concentrations of FT3 and FT4 thyroid hormones and thyroid gland histology between the control and exposure group of TCEP
	Metric 6:	Randomized Allocation	Low	random dosing was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	exposure preparation was well documented. Each kestrel ate one cockerel per day that had been injected with 50 "L of a safflower oil solution containing TCEP. Dosage was calculated by weight to ensure consistent weight between the flame retardants used int he study
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were reported
	Metric 9:	Measurement of Test Substance Concentration	High	actual measured concentrations were reported
	Metric 10:	Exposure Duration and Frequency	High	exposure duration (21 days) was long enough to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	authors used only one test concentration, normalized by body weight, across multiple chemicals so dosage spacing is not relevant in this case
	Metric 12:	Testing at or Below Solubility Limit	N/A	dietary dose was administered via vegetable oil carrier, therefore the solubility was not relevant.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately described and appropriate
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	no description of acclimatization process was provided
	Metric 15:	Number of Organisms and Replicates per Group	Low	7 individuals for each chemical with no replication. Given that the intention was to characterize the comparative effects of the test material and not to calculate a dose response, this is sufficient for statistical analysis

Continued on next page . . .

. . . continued from previous page

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	Other (please specify below) (Thyroid Function)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	test conditions were adequate and reasonably well documented
	Metric 17:	Outcome Assessment Methodology	High	methods were adequate to characterize the comparative toxicity of several related chemicals at the same exposure concentration
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	differences between the chemicals were compared for each treatment to vehicle-treated controls by repeated measures (RM) analysis of variance (ANOVA). Calculations were not provided.
	Metric 22:	Reporting of Data	High	data was reported adequately
	Metric 23:	Explanation of Unexpected Outcomes	High	no effects were not unexpected, explanation was adequate

Additional Comments: None

Overall Quality Determination High

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	identified in text and abstract
	Metric 2:	Test Substance Source	Low	TCEP was purchased from Sigma-Aldrich but not analytically verified
	Metric 3:	Test Substance Purity	High	purity reported as > 97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative control of safflower oil (the delivery medium) was used
	Metric 5:	Negative Control Response	High	No reported mortalities in the negative controls
	Metric 6:	Randomized Allocation	Low	Random dosing was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	exposure preparation was well documented. Each kestrel ate one cockerel per day that had been injected with 50 "L of a safflower oil solution containing TCEP. Dosage was calculated by weight to ensure consistent weight between the flame retardants used int he study.
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were reported
	Metric 9:	Measurement of Test Substance Concentration	High	actual measured concentrations were reported
	Metric 10:	Exposure Duration and Frequency	High	exposure duration (21 days) was long enough to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	authors used only one test concentration, normalized by body weight, across multiple chemicals so dosage spacing is not relevant in this case
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary dose was administered via vegetable oil carrier, therefore the solubility was not relevant.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	organisms were adequately described and appropriate
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	no description of acclimatization process was provided
	Metric 15:	Number of Organisms and Replicates per Group	Low	7 individuals for each chemical with no replication. Given that the intention was to characterize the comparative effects of the test material and not to calculate a dose response, this is sufficient for statistical analysis
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	test conditions were adequate and reasonably well documented

Continued on next page . . .

. . . continued from previous page

Study Citation:	Fernie, K. J., Palace, V., Peters, L. E., Basu, N., Letcher, R. J., Karouna-Renier, N. K., Schultz, S. L., Lazarus, R. S., Rattner, B. A. (2015). Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. Environmental Science and Technology 49(12):7448-7455.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Falco sparverius*; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5353113

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	methods were adequate to characterize the comparative toxicity of several related chemicals at the same exposure concentration
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies were reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	effects were compared for each treatment to vehicle-treated controls by nonparametric analysis of variance (ANOVA) and Repeated Measures ANOVA on ranked data using SAS 9.3. Calculations were not provided.
	Metric 22:	Reporting of Data	High	data was reported adequately
	Metric 23:	Explanation of Unexpected Outcomes	High	low TCEP levels in organisms was given possible explanations

Additional Comments: None

Overall Quality Determination High

Study Citation:	Stauffer Chem Co, (1981). Toxicology reports on FYROL FR-2 (volume I - II) with attachments and cover letter dated 020381. 8100271#88-8100271.
Duration:	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus domesticus*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5165206

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	Fyrol CEF. Lot f 3587-2-1, was identified as the test chemical with no other identifiers (e.g., CASRN, structure, formula). Online search shows FYROL CEF as a synonym for Tris(2-chloroethyl) phosphate CAS 119-96-8. However, Fyrol CEF is also listed as a synonym for at least one other CASRN.
	Metric 2:	Test Substance Source	High	Source reported as Specialty Chemical Division
	Metric 3:	Test Substance Purity	Low	Purity not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative and positive controls used.
	Metric 5:	Negative Control Response	High	Negative response for positive and negative controls.
	Metric 6:	Randomized Allocation	Low	Allocation method not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Reported details were limited
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Method of measuring exposure concentration not reported.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was adequate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	Only a single exposure level for test with no response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dosage administered orally.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Only age of chickens reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation or pretreatment not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organism per group and replicates were appropriate for test.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions not reported.
	Metric 17:	Outcome Assessment Methodology	Low	Response to treatment was zero.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Stauffer Chem Co, (1981). Toxicology reports on FYROL FR-2 (volume I - II) with attachments and cover letter dated 020381. 8100271#88-8100271.
Duration:	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus domesticus*; Adult
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5165206

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were consistent.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no differences were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Qualitative score for behavior were summed.
	Metric 22:	Reporting of Data	High	All outcomes reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2; Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified by name and CAS #
	Metric 2:	Test Substance Source	Low	Test substance source was reported as Sigma-Aldrich, but it did not appear to be analyzed by the performing laboratory
	Metric 3:	Test Substance Purity	High	Test substance purity was 98.95%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	All treated groups were compared to a DMSO vehicle controls.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported and adequate. Untreated nematodes reached L4 stage in growth.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	DMSO solvent was used in the preparation of the test media stock solutions, but little other information was provided on preparation. The experimental system was 96 well plates.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. 48h exposure in 96 well plates with 50 nematodes per well.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and suitable for the study type–48h exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	12 exposure groups were reported with a concentration range from 1uM to 200uM. Found in the supplementary material.
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO solvent concentration was appropriate at 0.1% (v/v)
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect. 50 nematodes per well with at least 3 replicates of each concentration according to the supplementary material.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Behl, M., Hsieh, J. H., Shafer, T. J., Mundy, W. R., Rice, J. R., Boyd, W. A., Freedman, J. H., Hunter, E. S., Jarema, K. A., Padilla, S., Tice, R. R. (2015). Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicology and Teratology 52(Pt B):181-193.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2; Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3479540

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Boyd et al 2009 was cited for organism maintenance. Kept at 20 C and fed E.coli, otherwise little other housing information was provided.
	Metric 17:	Outcome Assessment Methodology	Medium	Rice et al 2014 was cited for assessment methodology. Some details were provided in the paper– organisms' gross morphology and developmental stage were assessed as well as size
	Metric 18:	Consistency of Outcome Assessment	High	Organisms were assessed 48h after exposure using gross morphology and developmental stage. Biosort was used to assess size and number of organisms
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest. Data was provided in the supplementary material. Control response was described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP (tris(2-chloroethyl)
	Metric 2:	Test Substance Source	High	Purchased from Sigma Aldrich Chemical Co
	Metric 3:	Test Substance Purity	High	98.95% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors only reported two positive control groups used, containing chlorpyrifos and "aromatic OPFR TOCP".
	Metric 5:	Negative Control Response	High	No toxicity response from the positive control groups used.
	Metric 6:	Randomized Allocation	Low	How organisms were allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study did not comment on whether agar plates or well-plates were sealed.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent for all groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at beginning of exposure, but not at the end of the exposure period.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Number exposure groups and frequency were appropriate for other tested chemicals, however no adverse effects were reported for TCEP.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting were insufficient to determine if solubility limits were exceeded.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described and source provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	pretreatments and acclimation periods were adequately described.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of test organisms and replicates were adequate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test organism environmental conditions were acceptable.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.

Continued on next page . . .

...continued from previous page

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Assessments were consistent across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.
	Metric 22:	Reporting of Data	Low	Test results for exposure groups were reported in poorly rendered, graphs and only summarized cursorily in the text. No adverse outcomes were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No explanations was provided why TCEP was the only flame retardant that was inactive across all of the C.elegans endpoints.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP (tris(2-chloroethyl)
	Metric 2:	Test Substance Source	High	Purchased from Sigma Aldrich Chemical Co
	Metric 3:	Test Substance Purity	High	98.95% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors only reported two positive control groups used, containing chlorpyrifos and "aromatic OPFR TOCP".
	Metric 5:	Negative Control Response	High	No toxicity response from the positive control groups used.
	Metric 6:	Randomized Allocation	Low	How organisms were allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study did not comment on whether agar plates or well-plates were sealed.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent for all groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at beginning of exposure, but not at the end of the exposure period.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Number exposure groups and frequency were appropriate for other tested chemicals, however no adverse effects were reported for TCEP.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting were insufficient to determine if solubility limits were exceeded.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described and source provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	pretreatments and acclimation periods were adequately described.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of test organisms and replicates were adequate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test organism environmental conditions were acceptable.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessments were consistent across groups

Continued on next page . . .

. . . continued from previous page

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.
	Metric 22:	Reporting of Data	Low	Test results for exposure groups were reported in poorly rendered, graphs and only summarized cursorily in the text. No adverse outcomes were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No explanations was provided why TCEP was the only flame retardant that was inactive across all of the C.elegans endpoints.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	TCEP (tris(2-chloroethyl)
	Metric 2:	Test Substance Source	High	Purchased from Sigma Aldrich Chemical Co
	Metric 3:	Test Substance Purity	High	98.95% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors only reported two positive control groups used, containing chlorpyrifos and "aromatic OPFR TOCP".
	Metric 5:	Negative Control Response	High	No toxicity response from the positive control groups used.
	Metric 6:	Randomized Allocation	Low	How organisms were allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Study did not comment on whether agar plates or well-plates were sealed.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent for all groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at beginning of exposure, but not at the end of the exposure period.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Number exposure groups and frequency were appropriate for other tested chemicals, however no adverse effects were reported for TCEP.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting were insufficient to determine if solubility limits were exceeded.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described and source provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	pretreatments and acclimation periods were adequately described.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of test organisms and replicates were adequate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test organism environmental conditions were acceptable.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessments were consistent across groups

Continued on next page . . .

. . . continued from previous page

Study Citation:	Behl, M., Rice, J. R., Smith, M. V., Co, C. A., Bridge, M. F., Hsieh, J. H., Freedman, J. H., Boyd, W. A. (2016). Editor's highlight: Comparative toxicity of organophosphate flame retardants and polybrominated diphenyl ethers to Caenorhabditis elegans. Toxicological Sciences 154(2):241-252.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Bristol N2 (wild-type); Larvae
Health Outcome:	Behavioral
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	3975281

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.
	Metric 22:	Reporting of Data	Low	Test results for exposure groups were reported in poorly rendered, graphs and only summarized cursorily in the text. No adverse outcomes were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No explanations was provided why TCEP was the only flame retardant that was inactive across all of the C.elegans endpoints.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name and CASRN were reported.
	Metric 2:	Test Substance Source	High	Sourced from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	All chemicals were Analytical Grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was K Medium as were the rest of the treatments.
	Metric 5:	Negative Control Response	High	Body length of control reported in Figure 2, A1; seems appropriate for baseline as low concentration TCEP treatments had similar body lengths.
	Metric 6:	Randomized Allocation	Low	No mention of how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	"TCEP solutions were prepared in K-medium and the control groupis K-medium. Worms were exposed to a series of concentrations of TCEP (50, 250, 500, 750, 1000 mg in 24-well plates." Post exposure set up adequately described.
	Metric 8:	Consistency of Exposure Administration	Medium	Test organisms exposed for 3 days in 24-well plates. Lighting and other incubation conditions not described.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	Medium	Unclear if 3 day exposure duration with endpoints taken immediately post-exposure is sufficient for determining impact on body length.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure covered a wide range with good spacing, 0-1000 mg/L.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Sigma Aldrich states TCEP solubility in water is 50 mg/mL (50 g/L); 1000 mg/L (1 g/L), the high dose, would be far under the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Good description of C. elegans and how test organisms were obtained. "All strains of C. elegans were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA), and maintained in terms of standard protocols as previously described."
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Authors did not state if organisms were acclimated to 24-well plates prior to chemical exposure.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Sufficient to establish an adequate response. 50-100 worms per exposure concentration, experiments performed in quadruplet.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Details on total volume per well (assuming 500 uL) as well as temperature and lighting during exposure in 24-well plates lacking.
	Metric 17:	Outcome Assessment Methodology	High	Authors reported how lengths were assessed: "After being exposed to TCEP (0, 50, 250, 500, 750, 1000 mg/L)...for 3 d, worms were washed three times with M9 buffer, and then transferred to agar-padded slides and sealed with a coverslip which were immobilized with 100 mM sodium azide. Then body lengths of nematodes were measured using an imaging system."
	Metric 18:	Consistency of Outcome Assessment	High	No mention of any inconsistencies.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences of any variables that would alter the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated issues were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"All data were expressed as mean ± Standard Deviation (SD). Mean differences between treated groups and controls were determined by one-way analysis of variance (ANOVA), followed by Dunnett"s test. A p-value of less than 0.05 was considered significant."
	Metric 22:	Reporting of Data	High	Data for control and treatment groups were reported graphically with standard deviation (Figure 2, A1) and in text form.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes, deviations were reasonable.

Additional Comments: The form 3 for biomarker endpoint applies to GFP expression in BZ555 and YFP expression in NL5901 strains. The form 3 for neurological endpoint applies to both local movement and locomotor assays. Well reported study.

Overall Quality Determination High

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name and CASRN were reported.
	Metric 2:	Test Substance Source	High	Sourced from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	Percent purity not reported however chemicals stated to be Analytical Grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was K Medium as were the rest of the treatments.
	Metric 5:	Negative Control Response	Medium	Control response of near 100% survival, from graph. However, it was unclear if this response was scaled to 100% of control survival.
	Metric 6:	Randomized Allocation	Low	No mention of how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	"TCEP solutions were prepared in K-medium and the control groupis K-medium. Worms were exposed to a series of concentrations of TCEP (50, 250, 500, 750, 1000 mg in 24-well plates." Post exposure set up adequately described.
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing to suggest exposure was inconsistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations not measured but no indication that measured concentrations should deviate from nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	Sufficient duration (3 days) to obtain mortality over time post-exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure covered a wide range with good spacing, 0-1000 mg/L.
	Metric 12:	Testing at or Below Solubility Limit	High	Sigma Aldrich states TCEP solubility in water is 50 mg/mL (50 g/L); 1000 mg/L (1 g/L), the high dose, would be far under the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Good description of C. elegans and how test organisms were obtained. "All strains of C. elegans were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA), and maintained in terms of standard protocols as previously described."
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Authors did not state if test organisms were acclimated to 24-well plates prior to chemical exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Sufficient to establish an adequate response. 50-100 worms per exposure concentration, experiments performed in quadruplet.
Domain 5: Outcome Assessment				

Continued on next page . . .

. . . continued from previous page

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	No details on physical conditions of test organisms
	Metric 17:	Outcome Assessment Methodology	High	Reported how mortalities were assessed (every 2 days) over time post exposure.
	Metric 18:	Consistency of Outcome Assessment	High	No mention of any inconsistencies.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No mention of any variables that would alter the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated issues were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Percentages were reported as well as a mention of significant difference. Statistics for lifespan assay sufficiently described in cited methods Xu et al 2016 as well as under 'Statistical Analysis' section.
	Metric 22:	Reporting of Data	High	Data for control and treatment groups were reported graphically (Figure 2, C1) and in text form.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes, deviations were reasonable.

Additional Comments: The form 3 for biomarker endpoint applies to GFP expression in BZ555 and YFP expression in NL5901 strains. The form 3 for neurological endpoint applies to both local movement and locomotor assays. Well reported study.

Overall Quality Determination Medium

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; BZ555; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name and CASRN were reported.
	Metric 2:	Test Substance Source	High	Sourced from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	All chemicals were Analytical Grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was K Medium as were the rest of the treatments.
	Metric 5:	Negative Control Response	High	Control responses shown in Figure 6G and 7F and seem reasonable.
	Metric 6:	Randomized Allocation	Low	No mention of how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	"TCEP solutions were prepared in K-medium and the control groupis K-medium. Worms were exposed to a series of concentrations of TCEP (50, 250, 500, 750, 1000 mg in 24-well plates." Post exposure set up adequately described.
	Metric 8:	Consistency of Exposure Administration	Medium	Test organisms exposed for 3 days in 24-well plates. Lighting and other incubation conditions not described.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	High	3 day exposure period should be sufficient for GFP and YFP expression in the transgenic strains.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure covered a wide range with good spacing, 0-1000 mg/L.
	Metric 12:	Testing at or Below Solubility Limit	High	Sigma Aldrich states TCEP solubility in water is 50 mg/mL (50 g/L); 1000 mg/L (1 g/L), the high dose, would be far under the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Good description of C. elegans and how test organisms were obtained. "All strains of C. elegans were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA), and maintained in terms of standard protocols as previously described."
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors did not state whether organisms acclimated to 24-well plates prior to exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	At least 30 nematodes per group analyzed for GFP expression or YFP expression.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; BZ555; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Details on total volume per well (assuming 500 uL) as well as temperature and lighting during exposure in 24-well plates lacking.
	Metric 17:	Outcome Assessment Methodology	High	Analysis of PDat-1 in dopamine neurons via GFP tagging and alpha-synuclein in muscles via YFP tagging adequately described. Established transgenic strains utilized for these experiments.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment methodology adequately described by authors as well as cited references.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No mention of any variables that would alter the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"All data were expressed as mean ± Standard Deviation (SD).Mean differences between treated groups and controls weredetermined by one-way analysis of variance (ANOVA), followedby Dunnett"s test. A p-value of less than 0.05 was consideredsignificant."
	Metric 22:	Reporting of Data	High	Data for GFP expression in dopamine neurons (in BZ555 strain) shown in Figure 6 and data for YFP expression in muscles (in NL5901 strain) shown in Figure 7 as well as described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes, deviations were reasonable.

Additional Comments: The form 3 for biomarker endpoint applies to GFP expression in BZ555 and YFP expression in NL5901 strains. The form 3 for neurological endpoint applies to both local movement and locomotor assays. Well reported study.

Overall Quality Determination High

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Neurological
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name and CASRN were reported.
	Metric 2:	Test Substance Source	High	Sourced from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	All chemicals were Analytical Grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was K Medium as were the rest of the treatments.
	Metric 5:	Negative Control Response	High	Control responses in local movement and locomotor assays shown in Figures 3, 4, and 5 seem reasonable (i.e. similar to responses shown in low TCEP concentration treatments).
	Metric 6:	Randomized Allocation	Low	No mention of how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test set up described in 24-well plates as well as in cited references for NGM plates (Tsalik and Hobert 2003; Donnelly et al 2013; Gallagher et al 2013)
	Metric 8:	Consistency of Exposure Administration	High	Test organisms exposed to TCEP 3 days prior to local movement and locomotor assays as stated in Figure 3 and Figure 4.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was 3 days which should be sufficient for establishing effects on local movement and locomotion.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure covered a wide range with good spacing, 0-1000 mg/L
	Metric 12:	Testing at or Below Solubility Limit	High	Sigma Aldrich states TCEP solubility in water is 50 mg/mL (50 g/L); 1000 mg/L (1 g/L), the high dose, would be far under the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Good description of C. elegans and how test organisms were obtained. "All strains of C. elegans were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA), and maintained in terms of standard protocols as previously described."
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms acclimated briefly before local movement and locomotor assays in NGM plates. Authors did not state whether test organisms were acclimated to 24-well plate prior to chemical exposure.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Neurological
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Sufficient to establish an adequate response. 50-100 worms per exposure concentration in 24-well plates, experiments performed in quadruplet.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Details on total volume per well (assuming 500 uL) as well as temperature and lighting during exposure in 24-well plates lacking. Conditions in NGM plates adequately described by authors as well as cited references.
	Metric 17:	Outcome Assessment Methodology	High	Authors adequately described how local movement and locomotion was assessed via text and cited references.
	Metric 18:	Consistency of Outcome Assessment	High	No mention of any inconsistencies in any of the assessments.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No mention of any variables that would alter the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	"All data were expressed as mean ± Standard Deviation (SD).Mean differences between treated groups and controls weredetermined by one-way analysis of variance (ANOVA), followedby Dunnett"s test. A p-value of less than 0.05 was consideredsignificant."
	Metric 22:	Reporting of Data	High	Data for local movement shown in Figure 3 and data for locomotion shown in Figures 4 and 5 as well as described in text.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes, deviations were reasonable.

Additional Comments: The form 3 for biomarker endpoint applies to GFP expression in BZ555 and YFP expression in NL5901 strains. The form 3 for neurological endpoint applies to both local movement and locomotor assays. Well reported study.

Overall Quality Determination High

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Name and CASRN were reported.
	Metric 2:	Test Substance Source	High	Sourced from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	Percent purity not reported but all chemicals were Analytical Grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was K Medium as were the rest of the treatments.
	Metric 5:	Negative Control Response	Medium	Control response of near 100% survival, from graph; however, not clear if control survival was scaled to 100% for the purposes of the graph.
	Metric 6:	Randomized Allocation	Low	No mention of how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	TCEP prepared in K medium, 24-well plate set up adequately described.
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing to suggest exposure was inconsistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	High	Sufficient duration to obtain a good dose response; 1, 3, 6 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure covered a wide range with good spacing, almost two orders of magnitude.
	Metric 12:	Testing at or Below Solubility Limit	High	Sigma Aldrich states TCEP solubility in water is 50 mg/mL (50 g/L); 1000 mg/L (1 g/L), the high dose, would be far under the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Good description of C. elegans and how test organisms were obtained. "All strains of C. elegans were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA), and maintained in terms of standard protocols as previously described."
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Unclear if organisms were acclimated to 24-well plates prior to treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Adequate for endpoint determinations.

Continued on next page . . .

. . . continued from previous page

Study Citation:	Xu, T., Li, P., Wu, S., Lei, L., He, D. (2017). Tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloropropyl) phosphate (TCPP) induce locomotor deficits and dopaminergic degeneration in Caenorhabditis elegans. Toxicology Research 6(1):63-72.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Wild Type (Bristol, N2); Larvae
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469475

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Details on total volume per well (assuming 500 uL) as well as temperature and lighting during exposure in 24-well plates lacking.
	Metric 17:	Outcome Assessment Methodology	High	Reported how mortalities were assessed-unresponsive to gentle needle probe.
	Metric 18:	Consistency of Outcome Assessment	High	No mention of any inconsistencies.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No mention of any variables that would alter the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated issues were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Confidence intervals were included with LC50 values. "The median lethal concentrations (LC50) of TCEP and TCPP were determined bylinear regression analysis with Graphpad Prism."
	Metric 22:	Reporting of Data	High	LC50 values for 1, 3, and 6 day lethality tests were reported in text and dose responses were shown in graphically. Well documented.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes, deviations were reasonable.

Additional Comments: The form 3 for biomarker endpoint applies to GFP expression in BZ555 and YFP expression in NL5901 strains. The form 3 for neurological endpoint applies to both local movement and locomotor assays. Well reported study.

Overall Quality Determination High

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	positively identified in abstract and main body
	Metric 2:	Test Substance Source	Low	Obtained from TCI development Company, not analytically verified
	Metric 3:	Test Substance Purity	High	percent purity reported as >97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used. no chemical control was used
	Metric 5:	Negative Control Response	High	Mortality was less than 5% across treatments. Treatments were significantly different than controls.
	Metric 6:	Randomized Allocation	Low	no mention of random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	well documented experimental set up
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical treatment levels were not measured
	Metric 10:	Exposure Duration and Frequency	High	exposure duration followed guidelines and was sufficient to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	exposure concentration range was sufficient to assess outcomes
	Metric 12:	Testing at or Below Solubility Limit	Medium	no mention of how chemical dispersion occurred, this is a terrestrial study not sure if solubility is an issue for this
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well documented organism acclimatization and pretreatment procedure
	Metric 15:	Number of Organisms and Replicates per Group	Medium	organism numbers sufficient to assess outcome
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	well documented and adequate test conditions
	Metric 17:	Outcome Assessment Methodology	Medium	subsampling periodically for mortality is not the recommended method

Continued on next page . . .

. . . continued from previous page

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies in the assessment were noted
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables in the assessment were noted
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were noted in the assessment
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequate and reported in section 2.8
	Metric 22:	Reporting of Data	Low	no actual values for mortality were reported
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	positively identified in abstract and main body
	Metric 2:	Test Substance Source	Low	Obtained from TCI development Company, not analytically verified
	Metric 3:	Test Substance Purity	High	percent purity reported as >97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used. No chemical control was used
	Metric 5:	Negative Control Response	High	Mortality was less than 5% across treatments. Treatments were significantly different than controls.
	Metric 6:	Randomized Allocation	Low	no mention of random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	well documented experimental set up
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical treatment levels were not measured
	Metric 10:	Exposure Duration and Frequency	High	exposure duration followed guidelines and was sufficient to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	exposure concentration range was sufficient to assess outcomes
	Metric 12:	Testing at or Below Solubility Limit	Medium	no mention of how chemical dispersion occurred, this is a terrestrial study not sure if solubility is an issue for this
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well documented organism acclimatization and pretreatment procedure
	Metric 15:	Number of Organisms and Replicates per Group	Medium	organism numbers sufficient to assess outcome
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	well documented and adequate test conditions
	Metric 17:	Outcome Assessment Methodology	High	assessment methods were straightforward
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies in the assessment were noted

Continued on next page . . .

. . . continued from previous page

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables in the assessment were noted
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were noted in the assessment
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical significance compared to control reported in Figure 1.
	Metric 22:	Reporting of Data	High	growth rate values were reported reasonably well
	Metric 23:	Explanation of Unexpected Outcomes	Medium	a trend was reported but no explanation for a mid treatment level relative decrease

Additional Comments: None

Overall Quality Determination High

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Neurological
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	positively identified in abstract and main body
	Metric 2:	Test Substance Source	Low	Obtained from TCI development Company, not analytically verified
	Metric 3:	Test Substance Purity	High	percent purity reported as >97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used. No chemical control was used
	Metric 5:	Negative Control Response	High	Mortality was less than 5% across treatments. Treatments were significantly different than controls.
	Metric 6:	Randomized Allocation	Low	no mention of random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	well documented experimental set up
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical treatment levels were not measured
	Metric 10:	Exposure Duration and Frequency	High	exposure duration followed guidelines and was sufficient to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	exposure concentration range was sufficient to assess outcomes
	Metric 12:	Testing at or Below Solubility Limit	Medium	no mention of how chemical dispersion occurred, this is a terrestrial study not sure if solubility is an issue for this
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well documented organism acclimatization and pretreatment procedure
	Metric 15:	Number of Organisms and Replicates per Group	Medium	organism numbers sufficient to assess outcome
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	well documented and adequate test conditions
	Metric 17:	Outcome Assessment Methodology	High	assessment methods were straightforward
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies in the assessment were noted

Continued on next page . . .

. . . continued from previous page

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Neurological
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables in the assessment were noted
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were noted in the assessment
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	author noted what statistics were applied, gene transcription, GSH and AChE did not follow a clear dose response
	Metric 22:	Reporting of Data	High	Transcriptions, GSH levels and AChE levels rate values were reported reasonably well
	Metric 23:	Explanation of Unexpected Outcomes	High	trends were reported ando explanations were provided for results not following trends

Additional Comments: None

Overall Quality Determination High

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Gastrointestinal
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	positively identified in abstract and main body
	Metric 2:	Test Substance Source	Low	Obtained from TCI development Company, not analytically verified
	Metric 3:	Test Substance Purity	High	percent purity reported as >97%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used. No chemical control was used
	Metric 5:	Negative Control Response	High	Mortality was less than 5% across treatments. Treatments were significantly different than controls.
	Metric 6:	Randomized Allocation	Low	no mention of random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	well documented experimental set up
	Metric 8:	Consistency of Exposure Administration	High	no inconsistencies were noted
	Metric 9:	Measurement of Test Substance Concentration	Low	chemical treatment levels were not measured
	Metric 10:	Exposure Duration and Frequency	High	exposure duration followed guidelines and was sufficient to assess outcomes
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	exposure concentration range was sufficient to assess outcomes
	Metric 12:	Testing at or Below Solubility Limit	Medium	no mention of how chemical dispersion occurred, this is a terrestrial study not sure if solubility is an issue for this
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	well documented organism characteristics
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well documented organism acclimatization and pretreatment procedure
	Metric 15:	Number of Organisms and Replicates per Group	Medium	organism numbers sufficient to assess outcome
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	well documented and adequate test conditions
	Metric 17:	Outcome Assessment Methodology	High	assessment methods were straightforward
	Metric 18:	Consistency of Outcome Assessment	High	no inconsistencies in the assessment were noted

Continued on next page . . .

. . . continued from previous page

Study Citation:	Yang, Y., Xiao, Y., Chang, Y., Cui, Y., Klobučar, G., Li, M. (2018). Intestinal damage, neurotoxicity and biochemical responses caused by tris (2-chloroethyl) phosphate and tricresyl phosphate on earthworm. Ecotoxicology and Environmental Safety 15878-86.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Gastrointestinal
Chemical:	Tris(2-chloroethyl) phosphate (TCEP)
HERO ID:	5469239

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variables in the assessment were noted
	Metric 20:	Outcomes Unrelated to Exposure	High	no unrelated outcomes were noted in the assessment
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	author noted what statistics were applied, intestinal damage and DNA breaks followed a reasonable dose response biochemical changes did not follow a clear dose response
	Metric 22:	Reporting of Data	High	intestinal damage, DNA breaks and biochemical changes were reported reasonably well
	Metric 23:	Explanation of Unexpected Outcomes	High	trends were reported and explanations were provided for results not following trends

Additional Comments: None

Overall Quality Determination High