# Regression Analysis of Exposure Response Data from Chronic Aquatic Toxicity Tests for Chlorpyrifos, Diazinon and Malathion

Gregory Elonen1, Timothy D. Dawson1, David R. Mount2, and Dale J. Hoff2

1Badger Technical Services, LLC.

6201 Congdon Blvd.

Duluth, MN 55804

2U.S. Environmental Protection Agency

Office of Research and Development

National Health and Environmental Research Laboratory

Mid-Continent Ecology Division

6201 Congdon Blvd.

Duluth, MN 55804

March 3, 2016

**1.0 INTRODUCTION**

This report summarizes analysis of effect endpoints from chronic toxicity tests with aquatic species exposed to chlorpyrifos, diazinon and malathion. Test results are presented as No and Lowest Observed Effect Concentration (NOEC/LOEC) values from hypothesis testing reported by the study authors, and as point estimates (EC5, EC10, EC20, and EC50) derived from regression analyses conducted by the authors of this report. These data analyses are a subset of those originally conducted for other purposes; the specific data presented here were extracted and compiled in this report at the request of staff from EPA’s Office of Pesticide Programs to support other analyses of toxicity data for chlorpyrifos, diazinon, and malathion.

Users of this information should recognize that analysis of toxicity test data can involve some degree of subjectivity, such as in the selection of regression models, or in decisions on how to analyze test data when they do not neatly conform to typical forms of exposure response relationships. The methods below describe the basic approach used in analyzing test data, but the results are not always the only interpretation that could be applied; users should determine whether the approaches used are appropriate for specific applications.

Section 2 describes the methods used to compile and analyze the toxicity data, while Section 3 provides the results and a brief discussion. Appendix A contains the guidance applied in selecting regression models, while Appendix B contains the grading criteria for assigning regression results to different quality categories. Appendix C is a tabular summary of detailed results, including NOEC/LOEC and ECx values for multiple endpoints within tests, and sources of the data. Appendix D provides graphs of all data sets showing the treatment level response data and the NOEC, LOEC, and EC20 values.

**2.0 DATA COMPILATION AND ANALYSIS METHODS**

The data used in this report were extracted from the AquaChronTox (ACT) database; this database is a compilation of chronic toxicity data for aquatic animals obtained from studies that would meet the definition of an acceptable chronic test under the 1985 Ambient Water Quality Criteria (AWQC) Guidelines (US EPA, 1985) and/or under the effects assessment framework applied for evaluating the ecological effects of pesticides within OPP. In general terms, this means life-cycle tests (including reproduction) with invertebrates, and life-cycle, partial life-cycle, or early life stage (ELS) tests with fish. To be included in ACT, the original test data must have been provided at the treatment level (a minimum of mean response in each treatment); replicate level data are captured if available, but are not required. Statistically determined No Observed Effect Concentration (NOEC; highest exposure concentration without statistically significant effect relative to control) and Lowest Observed Effect Concentration (LOEC; lowest exposure concentrations showing statistically significant effect relative to control) values were included from either the study authors or from later re-analysis (e.g., data evaluation records completed as part of USEPA risk assessments); NOEC/LOEC values were not re-evaluated as part of the current analysis.

The data sources for ACT included: 1) Data Evaluation Records (DERs) developed by OPP; 2) publications identified in Ambient Water Quality Criteria documents. Publications from the open literature were identified in the ECOTOX database and are referenced here by their ECOTOX identification number (cross referenced to traditional citation in the references); studies evaluated via DER are referenced by their MRID number. Tests using low purity (<85%) or formulated materials were not included. Each test was screened for some general quality/applicability criteria (e.g., test chemical characteristics, use of controls, test design, exposure conditions). Classification of a test by a DER as “core” (i.e. high quality data for risk assessment) or “supplemental” (i.e., studies considered to have one or more substantial flaws but from which useful information is available) was taken as evidence that characteristics of the test were generally appropriate. For the three chemicals covered in this report, data from twenty six chronic exposures were considered; eleven were from DERs and fifteen were from publications from Ambient Water Quality Criteria documents. Of the eleven studies with DERs, three were not included in these analyses. MRID 41422401, a malathion test with rainbow trout (*Oncorhynchus mykiss*), was not included because treatment level data were not available. MRID 40914801, a diazinon test on sheepshead minnows (*Cyprinodon variegatus*) was not included because the only response data from the test organism’s first generation was a reproductive response which was considered too limited to compare to other studies with additional endpoints. MRID 46867001, a diazinon test on fathead minnows (*Pimephales promelas*), was not included because the test methodology deviated substantially from standard protocol. Of the eight remaining tests covered by DERs, five were classified as core and three were classified as supplemental. If a test reported data for multiple durations of a particular biological variable, then the duration most closely matching ASTM testing guidelines was generally the only duration analyzed. For tests involving exposure over more than one life cycle, only data from the first life cycle are presented.

Regression analysis was used to estimate the exposure concentrations associated with 5%, 10%, 20%, and 50% reductions in performance relative to control response (the EC05, EC10, EC20, and EC50, respectively). Regression analysis was completed for each reported biological variable using the Toxicity Response Analysis Program (TRAP, Version 1.22; U.S. EPA, Mid-Continent Ecology Division, Duluth, MN: (<http://archive.epa.gov/med/med_archive_03/web/html/trap.html> ). The criteria used to select regression models for individual data sets are provided in Appendix A. Both negative and solvent control data were included in the regression analyses, regardless of how the study authors or reviewers utilized control values when determining levels of significance. In tests with pesticides, control treatments are generally reported as having either a concentration of zero, or a concentration below analytical detection limits; in these cases, control exposures were arbitrarily assigned a concentration equal to 10% of the lowest treatment (regression analysis based on log-transformed exposure concentrations precludes entering zero as an exposure concentration). As a general guideline, an EC20 was reported only if the highest exposure concentration caused an effect of ≥15 % relative to the control (i.e., the “no response” value for the biological variable, or Y0) estimate and the data indicated a plausible exposure response curve. An EC50 was reported only if the data indicated a reasonable exposure response curve and the highest exposure concentration caused an effect of at least 40% relative to the Y0 estimate. ECx values were also not reported if they were derived largely by interpolation between the lowest exposure concentration and the control. A more thorough explanation of the guidelines and how we applied them to these data are provided in Appendix A, Response Curve Modeling Rationale and Guidelines.

In the chronic tests evaluated, organism growth was sometimes reported as weight (wet or dry) and sometimes as length (or both).  This distinction is generally unimportant when conducting hypothesis-driven statistics, but when performing regression analysis to a specified level of effect (e.g., EC20), length and weight can be expected to yield different response curves, because weight reflects the combined effects of growth in three dimensions (length x width x height), but length reflects only one of these dimensions.  Thus, one expects a 20% reduction in length to equate to a much, much larger reduction in weight, making EC20s based on weight and EC20s based on length not directly comparable.

In tests evaluated here, weight was the preferred endpoint; if growth was assessed as both weight and length, regression analysis of weight data was given preference. If regressions were to be based on length, the length data were transformed by taking the cube of length (L3) before performing the regression analysis. This is based on the premise that for an object of fixed proportions and density, the mass of the object will increase with the cube of any one dimension. To test the appropriateness of this approach, we selected a subset of chronic tests in ACT for which both length and weight were reported. We then calculated EC20 values for weight, length, and length3 for the same data set, and compared the resulting EC20 values as shown in Figure 1. EC20s calculated based on weight and length3 showed a good correspondence, with the data distributed fairly evenly above and below the unity line with a small overall bias toward EC20s based on length3 being more sensitive (Figure 1 upper panel). In contrast, the plot comparing EC20s for weight and length (not cubed) showed that EC20 values based on length were consistently biased high relative to weight (Figure 1 lower panel), generally occurring at about 2x the EC20 for weight. We believe this analysis supports the use of length3 as a reasonable surrogate for weight data. In some cases we have also included regression analysis of length for comparison purposes but these were not used in the final analysis.

Another endpoint analyzed by regression analysis was biomass, which combines effects on weight and survival into a single endpoint. Biomass was calculated as the product of the reported weight (or L3 if weight wasn’t reported but length was) and the fraction of original organisms surviving for each treatment. Because NOEC or LOEC values for biomass were not reported and we did not calculate them post-hoc we used the lowest (most sensitive) endpoint of the NOEC and LOEC values associated with the underlying growth and survival responses for comparison with ECx values.

The degree to which different data sets support a robust regression analysis varies with a number of factors, such as the number of treatments showing partial effects, the degree of variability among controls and treatments with low levels of effect, and the monotonicity of the response curve. To a degree, this is reflected in the confidence limits associated with ECx values; however in some cases, ECx values can be reasonably inferred from data sets that do not support calculation of rigorous confidence limits. To provide some characterization of the relative uncertainty associated with different regression analyses, we developed a categorical score for each regression, assigning 1, 2 or 3 (called a TRAP quality score in this report, see Appendix B for narrative standards). While these scores are somewhat subjective, they provide a general indication of level of confidence that might be associated with particular values. The major factors influencing the assigned score parallel the major parameters of a exposure-response regression: a) how well the data demonstrate an unambiguous value under control or minimally stressful exposure; b) how well the response curve is anchored at higher levels of effect; and c) how well the data indicate the shape or slope of the range between minimal and maximal effect.

A score of 1 indicates that the test data yield a generally unambiguous exposure-response curve for most or all ECx (EC05 –EC50) values. A score of 2 indicates that there is useful information regarding ECx values, but there are some uncertainties associated with the reported values. A score of 3 indicates that the test data have characteristics that create significant ambiguity in determining an exposure-response curve or that the data did not indicate a level of effect sufficient for regression analysis.

**3.0 RESULTS AND DISCUSSION**

Table 1 summarizes the tests included and provides the NOEC, LOEC and EC20 values for the most sensitive endpoint. For determining the most sensitive endpoint based on regression analysis, only those endpoints with a TRAP quality score of 1 or 2 were included except for one study (figures 28a, 28b, 29a, Appendix D) where all TRAP quality scores received a value of 3. Appendix C provides a more detailed tabulation of results, including ECx values for all individual endpoints, reference information, and a cross reference to the detailed graphical results in Appendix D. Chlorpyrifos was the most frequently tested chemical reported with a total of fourteen tests involving eight different species, compared to five tests over four species for diazinon and four tests and three species for malathion. Graphical presentations of data for each test and endpoint are included in Appendix D, along with a table of the associated NOEC, LOEC, and ECx values.

Growth (length or weight) was most frequently the most sensitive variable, though both survival and growth were most sensitive in several instances. Biomass was the most sensitive endpoint using regression analysis in 9 of the studies examined. It should be noted that early life stage tests with fish do not include measurement of reproduction, which could influence the distribution of sensitivity among endpoints.

For the 23 chronic tests where comparisons could be made (Table 1), the EC20 was greater than the LOEC in 11 tests (48%), the EC20 was between the NOEC and LOEC 10 times (43%), and the EC20 was lower than the NOEC for two tests (9%). In the first of the two latter cases (chlorpyrifos and % Survival of *Menidia peninsulae*), it appears the EC20 was lower than the NOEC because of high variability in the control treatments, which reduced the power of the statistics and raised the NOEC up to a higher concentration farther out on the apparent response curve (see Figure 18a in Appendix D; note that this regression received a score of 2). In the second case (malathion and *Jordanella floridae*), the most sensitive endpoint was length3 (see Figure 51b in Appendix D; note that this regression received a score of 2). In this study, calculating the EC20 based on length3 increased the steepness of the exposure-response curve, and also increased the difference in response between the control and low treatments. In this instance, the placement of the EC20 is highly dependent on the appropriateness of the control value, as even concentrations below the EC20 showed length3 values that were near or beyond 20% effect, even though the response curve was relatively shallow. Nonetheless, in both cases where the EC20 was lower than the NOEC, the EC20 was not greatly lower than the NOEC, considerably less than a factor of 2.

A broader comparison of NOEC values to ECx values was completed by plotting NOEC values from this data compilation against the corresponding EC10, EC20, and EC50 values from Appendix C (Figure 2). This comparison included all endpoints for a test that yielded regressions that scored 1 or 2. Comparisons with indefinite NOEC values or unreported ECX values were excluded. In general, the EC10 values fell close to the NOEC values. An alternative expression is the ratio of the ECX to the NOEC (ECX/NOEC), for which a value of 1 would indicate equality and ratios above 1 would indicate ECX > NOEC (and vice versa). Median values (with 10th and 90th percentiles in parenthesis) for these ratios were 1.23 (0.76 – 2.8) for the EC10, 1.58 (0.89 – 5.2) for the EC20, and 3.27 (1.37 – 15.5) for the EC50. These ratios were developed from distributions with all endpoints combined; it is likely that ratios may co-vary to some degree with endpoint; for example, response slopes for weight (or length) tend to be shallower than those for survival, which would typically lead to higher EC50/NOEC ratios. In addition, endpoints with high inter-replicate variability would tend to suppress ECX/NOEC ratios because a higher level of effect would be required to establish statistical significance in the NOEC-LOEC determination.

**References**

ECOTOX 995, Hermanutz,R.O, 1978, Endrin and Malathion Toxicity to Flagfish (Jordanella floridae), Arch. Environ. Contam. Toxicol.7(2): 159-168.

ECOTOX 3750, McKenney,C.,Jr., E. Matthews, and D. Lawrence, 1981, Chronic Toxicity Testing and Physiological Studies. A. Chronic Toxicity Tests: Invertebrates. 1. Mysid Life-Cycle Toxicity Tests, Progress Report, FY 81, October 1, 1980 - September 30, 1981, U.S. EPA, Environmental Research Laboratory, Gulf Breeze, FL:62-73.

ECOTOX 4225, Goodman,L.R., D.J. Hansen, D.P. Middaugh, G.M. Cripe, and J.C. Moore, 1985, Method for Early Life-Stage Toxicity Tests Using Three Atherinid Fishes and Results with Chlorpyrifos, ASTM Spec. Tech. Publ.:145-154,

ECOTOX 5074, Hansen,D.J., and P.R. Parrish, 1977, Suitability of Sheepshead Minnows (Cyprinodon variegatus) for Life-Cycle Toxicity Tests, ASTM Spec. Tech. Publ.:117-126.

 ECOTOX 7769, Cripe,G.M., D.J. Hansen, S.F. Macauley, and J. Forester, 1986, Effects of Diet Quantity on Sheepshead Minnows (Cyprinodon variegatus) During Early Life-Stage Exposures to Chlorpyrifos, ASTM Spec. Tech. Publ.9:450-460.

ECOTOX 10687, Hermanutz,R.O., J.G. Eaton, and L.H. Mueller, 1985, Toxicity of Endrin and Malathion Mixtures to Flagfish (Jordanella floridae), Arch. Environ. Contam. Toxicol.14:307-314.

ECOTOX 11709, Hansen,D.J., L.R. Goodman, G.M. Cripe, and S.F. Macauley, 1986, Early Life-Stage Toxicity Test Methods for Gulf Toadfish (Opsanus beta) and Results Using Chlorpyrifos, Ecotoxicol. Environ. Saf.11(1): 15-22.

ECOTOX 12881, Goodman,L.R., D.J. Hansen, G.M. Cripe, D.P. Middaugh, and J.C. Moore, 1985, A New Early Life-Stage Toxicity Test Using the California Grunion (Leuresthes tenuis) and Results with Chlorpyrifos, Ecotoxicol. Environ. Saf.10(1): 12-21.

ECOTOX 15462, Jarvinen,A.W., and D.K. Tanner, 1982, Toxicity of Selected Controlled Release and Corresponding Unformulated Technical Grade Pesticides to the Fathead Minnow Pimephales promelas, Environ. Pollut. A.27(3): 179-195.

US EPA. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. United States Environmental Protection Agency. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. PB85-227049. National Technical Information Service, Springfield, VA.

Figure 1 – EC20 values based on length versus those based on weight. Upper panel shows EC20s based directly on length3; lower panel shows EC20s based on length. 



Figure 2 – Comparison of EC10, EC20, and EC50 values to NOEC values for data reported in Appendix C. Data restricted to comparisons from regressions with scores of 1 or 2. Multiple endpoints may be reported from the same test.

Table 1-- Summary of tests used in this report.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Appendix D, Page Number | Pollutant | Test Species | Endpoints Reported | Most Sensitive EndpointHypothesis Testing | Most Sensitive EndpointRegression Analysis | EC20 Relative to NOEC / LOEC Values |
| NOEC (ug/L) | LOEC (ug/L) | Endpoint | EC20 (ug/L) | Endpoint | EC20<NOEC | EC20 between | EC20>LOEC |
| 2,3,4 | Chlorpyrifos | *Americamysis bahia* | S,R,G | 0.002 | 0.004 | G | 0.015 | G |  |  | x |
| 5,6,7 | Chlorpyrifos | *Americamysis bahia* | S,R,G | 0.0046 | 0.010 | S,G, | 0.0061 | B |  | x |  |
| 8,9,10,11 | Chlorpyrifos | *Pimephales promelas* | S,R,G | 0.57 | 1.09 | S | 1.19 | S |  |  | x |
| 12,13 | Chlorpyrifos | *Pimephales promelas* | S,G | 1.6 | 3.2 | G | 3.0 | B |  | x |  |
| 14,15 | Chlorpyrifos | *Menidia menidia* | S,G | 0.28 | 0.48 | S,G | 0.33 | S, B |  | x |  |
| 16,17 | Chlorpyrifos | *Menidia beryllina* | S,G | 0.36 | 0.75 | G | 0.96 | B |  |  | x |
| 18,19 | Chlorpyrifos | *Menidia peninsulae* | S,G | 0.38 | 0.78 | S | 0.25 | S | x |  |  |
| 20,21 | Chlorpyrifos | *Leuresthes tenuis* | S,G | 0.30 | 0.63 | G | 0.32 | B |  | x |  |
| 22,23 | Chlorpyrifos | *Leuresthes tenuis* | S,G | 0.28 | 0.62 | G | 0.42 | B |  | x |  |
| 24,25 | Chlorpyrifos | *Opsanus beta* | S,G | <18 | 18 | G | 16 | G |  | x |  |
| 26,27 | Chlorpyrifos | *Opsanus beta* | S,G | 1.4 | 3.7 | G | 8.7 | G |  |  | x |
| 28,29 | Chlorpyrifos | *Cyprinodon variegatus* | S,G | 1.7 | 3.0 | G | >3.0\* | B |  |  | x |
| 30,31,32 | Chlorpyrifos | *Cyprinodon variegatus* | S,G | 3.1 | 7.2 | G | 15.3 | G |  |  | x |
| 33 | Chlorpyrifos | *Daphnia magna* | S,R | 0.04 | 0.08 | S,G | 0.05 | S |  | x |  |
| 34,35,36,37,38 | Diazinon | *Americamysis bahia* | S,R,G | 0.23 | 0.42 | G | 0.90 | B |  |  | x |
| 39 | Diazinon | *Daphnia magna* | R | 0.17 | 0.32 | R | 0.19 | R |  | x |  |
| 40,41 | Diazinon | *Pimephales promelas* | S,G | 50 | 90 | G | 140 | G |  |  | x |
| 42,43 | Diazinon | *Pimephales promelas* | S,G | <92 | 92 | G | 117 | G |  |  | x |
| 44,45,46 | Diazinon | *Cyprinodon variegatus* | S,G | 4.3 | 8.0 | G | 6.0 | B |  | x |  |
| 47,48,49 | Malathion | *Daphnia magna* | S,R,G | 0.06 | 0.10 | R | 0.19 | R |  |  | x |
| 50,51,52 | Malathion | *Jordanella floridae* | S,R,G | 8.6 | 10.9 | G | 6.5 | G | x |  |  |
| 53,54,55 | Malathion | *Jordanella floridae* | S,R,G | 13.8 | 18.5 | G | 18.7 | G |  |  | x |
| 56,57,58 | Malathion | *Cyprinodon variegatus* | S,R,G | 9 | 18 | S | 12 | S |  | x |  |

S=Survival G=Growth R=Reproduction B=Biomass

\*Less than 20% effect at highest exposure concentration.