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| Appendix B |
| **GUIDELINES FOR ASSESSING RELATIVE QUALITY OF TRAP ANALYSIS GRAPHS** |
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**Appendix B: Guidelines for Assessing Relative Quality of TRAP Analysis Graphs**

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The following is intended as a guide to assessing regression analysis plots produced by the Toxicity Response Analysis Program (TRAP) v1.22 software developed by Russell J. Erickson (U.S. EPA). TRAP software was used for regression modeling and ECx estimations of 23 aquatic chronic toxicity tests with chlorpyrifos, diazinon and malathion. These analyses resulted in 93 graphs of various growth, reproductive and survival endpoints for which data were available. As a means of assessing the relative quality/reliability of regression results, we evaluated the regressions according to several criteria and assigned a score of 1, 2 or 3. A score of 1 indicates that the test data yield a generally unambiguous exposure-response curve for most or all ECx (EC05 –EC50) values (exceptions noted on the table accompanying each plot). 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. These are general guidelines and were used in conjunction with professional judgement when evaluating these data. What follows is a narrative explaining the general graph qualities that were examined as well as some examples of different exposure-response curves to help clarify the data evaluation process.

**General Criteria for Evaluating TRAP Regression Analysis**

Four elements of these plots were examined in an effort to evaluate the quality of the regression. Deviations away from the stated ideal reduced the perceived quality/reliability resulting in a higher score. The more pronounced the deviation, or the more categories that showed deviations, the greater the impact on the score. The following four criteria were considered:

1) Response increases with exposure in an interpretable manner and with at least one response of at least 40% of the Y0 intercept value for estimation of an EC50, or 15% for estimation of an EC20.

2) Sufficient data points near the control or Y0 value to define a reasonable “no effect” plateau for the regression curve.

3) Variability within the exposure response curve does not create substantive uncertainty in defining ECx values.

4) Control responses that appear coherent with the overall response curve and support unambiguous definition of both “no effect” response values and ECx concentrations.

A graph that consistently followed the above criteria would receive a score of 1. If it deviated appreciably from at least one of these criteria it may be scored as a 2. If the deviation was even more pronounced in at least one category or there were deviations in multiple categories, then it would receive a score of 3. Based on guidelines found in Appendix C “Response Curve Modeling Rationale and Guidelines”, all ECx values are not necessarily reported for every endpoint regression. Only those values that seem reasonable to estimate with minimal extrapolation are reported (see criterion #1 above).

To better explain the application of relative quality scoring, a number of examples that focus on compliance and non-compliance to each one of these four guideline statements along with a short description of the scoring process for each case follows.

**Criterion 1: Response increases with exposure in an interpretable manner and shows sufficient response to support ECx estimation**.

If the data are not sufficiently different from the control values then an estimate of EC20 or EC50 values becomes more uncertain. Guidelines for minimum effect values to estimate various ECx values are stated in the “Response Curve Modeling Rationale and Guidelines” found in Appendix A. Below are three examples of this. Graph 1 below, is an example that followed all the criteria and scored a 1 (from Figure12b, Appendix D). As can be seen, the no effect plateau is established with three data points. The slope is also well defined with three data points that follow a predictable path and the effects of the highest concentration are greater than 40% different than the Y0 value. Throughout the concentration range there are also no substantial deviations from the regression line. Graph 2 below was given a score of 2. It shows effects on weight (image 18b) which had a maximum negative effect about 20% less than the Y0 value. TRAP provided an estimate of an EC50 for this plot but because its highest concentration effect was less than 40% of the Y0 value, a significant extrapolation was required for that estimation. The EC50 was therefore considered unreliable. Graph 3 below was given a score of 3. It shows effects on male weight (image 28b). The effects were less than 15% different from the Y0 value and therefore there is insufficient effect to confidently estimate EC20 or EC50 values because of the extensive extrapolation required.

NOEC

EC20

EC20

LOEC

NOEC

GRAPH 1 GRAPH 2

LOEC

NOEC

GRAPH 3

**Criterion 2: Sufficient data points near the control or Y0 value to define a reasonable “no effect” plateau for the regression curve.**

The preferred exposure response curve includes at least one exposure response similar to the values of the control treatment to reinforce the certainty in a level of no exposure response. This in turn provides greater confidence in ECx estimations. Deviations from a no exposure response increase the uncertainty of ECx estimations throughout a regression curve, this is especially true at the lower levels, i.e., EC05 and EC10 estimates. The three plots shown below all demonstrate varying degrees of deviation from this guideline and progressively higher scores (i.e. increasing uncertainty). The two lowest concentrations of Graph 4 (image 40a) have an exposure response very similar to the control value and scored a 1. The lowest concentration in graph 5 (image 25a) is below the control response making the estimations of an EC05 and EC10 quite uncertain. This graph scored a 2. The lowest concentration in graph 6 (image 36b) was considerably higher than either control response, it was given a score of 3.

NOEC

EC20

LOEC

LOEC

EC20

 **GRAPH 4 GRAPH 5**

NOEC

 GRAPH 6

**Criterion 3: Variability within the exposure response curve does not create substantive uncertainty in defining ECX values**

If there is substantial variability among treatments in the “no response” portion of the regression curve, it creates uncertainty as to whether ECx values for low levels of effect are reflective of true response. In the example shown below in Graph 7 (image 37a), there is good agreement among responses in the low concentration range. It was given a score of 1. Graph 8 (image 53a), shows a case where there is considerable variability in response among the lower treatments; response in the lowest exposure concentration is reduced by roughly 20%, creating uncertainty as to whether the response at the calculated EC20 is really different than background variability. This regression was scored as a 2. Graph 9 (image 36b) shows even greater variability than graph 8 in the two lowest treatments and scored a 3.

NOEC

EC20

EC20

LOEC

NOEC

GRAPH 7 GRAPH 8

NOEC

GRAPH 9

**Criterion 4: Control responses that appear coherent with the overall response curve and support unambiguous definition of both “no effect” and ECx concentrations**

4a: If more than one control response is reported then their effects should be reasonably similar. Graph 10 (image 20a) has reasonably close agreement between control responses, it scored a 1. Graph 11 (image 18a) is an example of a plot that did not have good control treatment agreement so the estimation of an EC05 or an EC10 value would be difficult to do with any degree of certainty. Due in part to this uncertainty this graph was scored as a 2. Graph 12 (image 5b) exhibits even greater disagreement between control effects than shown in graph 11, it received a score of 3.

NOEC

EC20

LOEC

LOEC

NOEC

EC20

GRAPH 10 GRAPH 11

LOEC



GRAPH 12

4b: Control values that are considerably lower than the values of other low concentrations make interpreting the Y0 value difficult. This may result in a higher score such as in the following examples. Graph 13 (image 47b) shows good control agreement and its mean value is similar to the low concentration value. It was given a score of 1. The mean control value shown in graph 14 (image 33a) is somewhat lower than four treatment values in the plot. It scored a 2. Graph 15 (image 33b) has a mean control response considerably lower than five of six treatments, and shows considerable spread in values across the control and low concentrations, making the appropriate value for Y0 highly uncertain. Because of the associated uncertainty this creates for determining appropriate ECx values, it scored a 3.

EC20

LOEC

NOEC

NOEC

EC20

LOEC

**GRAPH 13 GRAPH 14**

NOEC

LOEC

**GRAPH 15**