Appendix E. Analysis of chronic toxicity data and acute chronic ratios (ACRs) in support of deriving chronic HC5s: Acetylcholinesterase inhibitors

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1.0 BACKGROUND

The analysis described in this appendix is one of several conducted in support of developing a common methodology for assessing chemical effects on aquatic animals for use by the USEPA Office of Pesticide Programs (OPP) and the Office of Water (OW). Other appendices describe methods for estimating/predicting the acute toxicity values and/or the acute HC5, the 5th percentile in a species sensitivity distribution for acute toxicity. However, effects assessments by both OPP and OW also require estimation of chronic toxicity thresholds. This could include predicting chronic thresholds for specific organisms, or the estimation of the chronic HC5, the 5th percentile of a chronic species sensitivity distribution.

A common approach for extrapolating from acute to chronic toxicity is the Acute-Chronic Ratio (ACR), which is calculated as the ratio of the acute effect concentration to a chronic effect concentration, generally for the same chemical and organism. Both OPP and OW currently use ACRs in some contexts, generally as a "within chemical" extrapolation tool, meaning that an ACR developed for a chemical tested with one or more species is used to estimate chronic effect thresholds for other organisms exposed to the same chemical. The underlying premise of the ACR is that the combination of toxicokinetics and toxicodynamics that determine the separation of acute and chronic effect concentrations has some degree of commonality among organisms (or sub-groups thereof) and can therefore serve as a basis for extrapolating chronic thresholds for organisms having only acute toxicity data.

As an example, EPA's guidelines for deriving aquatic life criteria (ALC)(USEPA 1985; referred to as the "1985 Guidelines") describe ACRs as one method for deriving the Final Chronic Value (FCV), which is intended to represent a theoretical 5th percentile of chronic toxicity for a distribution of aquatic species – essentially, the chronic HC5. The method in the 1985 Guidelines requires that acute and chronic toxicity data are available to calculate ACRs for a minimum of one fish, one invertebrate, and one acutely sensitive freshwater organism. A Final Acute-Chronic Ratio (FACR) is then calculated as the geometric mean of the available ACRs, or by other means as warranted by the data. For example, if the ACRs tend to decrease as acute sensitivity decreases, then the FACR may be calculated as the geometric mean of ACRs only for acutely sensitivity species. The Final Acute Value (FAV; the theoretical 5th percentile of the acute species sensitivity distribution) is then divided by the FACR to derive the FCV.

The FAV and FCV under the 1985 guidelines represent estimates of the acute HC5 and chronic HC5, and therefore present a case example of using ACRs to estimate the chronic HC5. To generalize this case, we can represent the approach as:

$$\text{chronic HC5} = (\text{acute HC5}) / ACR_{HC5}$$

where ACR_{HC5} refers specifically to an ACR derived for the purpose of relating an acute HC5 to a chronic HC5. In the case of the 1985 guidelines, the FACR = ACR_{HC5}. As outlined above, calculation of the FACR under the 1985 Guidelines requires the availability of paired acute and chronic toxicity data for a minimum group of organisms exposed to the same chemical. However, cases exist where OPP or OW may wish to estimate chronic effect thresholds for

chemicals with limited or no chronic toxicity data available; in such cases, an appropriate ACR must be developed from other data.

As part of the Great Lake Water Quality Initiative (GLI), a method was proposed to use "default" values in the calculation of an ACR_{HC5} (referred to in the methodology as a "Secondary Acute-Chronic Ratio" or SACR). The basis for this default value was an analysis by Host *et al.* (1995), who developed a distribution of FACR values from all ALC documents available at the time. The 80^{th} percentile of this distribution (18), was then adopted under the GLI as a default ACR value to be used in lieu of measured ACRs. Calculation of the SACR uses a combination of available measured ACRs and the default value of 18. If two measured ACRs are available, the SACR is the geometric mean of 18 and the two measured ACRs; if one measured ACR is available, the SACR is the geometric mean of 18, 18, and the measured ACR. If no chronic data are available, then the SACR is simply 18.

The FACR distribution compiled by Host *et al.* (1995) combined FACRs from freshwater ALC across all chemical types. TenBrook et al. (2010) took a similar approach, but used FACR data only for pesticides (combining pesticides of different types). The 80^{th} percentile of that distribution was 12.4, and TenBrook et al. (2010) recommended application of this value in a manner parallel to the SACR calculation under the GLI.

In the ACR_{HC5} estimation methods described above, default ACRs were derived from distributions of chemicals with multiple mechanisms of action. In the context of the current effort, it was hypothesized that the ACR_{HC5} approach could be refined by analyzing ACRs in the context of MOAs. The working assumption was that the distributional characteristics of ACRs would be related to the MOA for a chemical, meaning that particular MOAs might lead to consistently higher or lower ACRs compared to averages obtained across multiple MOAs. MOA-specific ACRs would logically extend also to the possibility of particular taxa having higher or lower ACRs because of the intersection of their physiology and the way in which the chemical elicits adverse effects.

This appendix analyzes ACR data for pesticides whose primary MOA is the inhibition of acetylcholinesterase (AChE); this includes two primary pesticide groups, organophosphates and carbamates. While focusing specifically on AChE-inhibiting chemicals, this analysis is also intended as demonstrative of analyses that might be used for developing MOA-specific ACRs for other groups of chemicals in the future.

2.0 DATA COMPILATION AND ANALYSIS

Chronic toxicity data were extracted from a database developed by USEPA called "AquaChronTox". 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 Guidelines; 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. Sources of data included a previous chronic toxicity data base compiled by Glen Thursby (USEPA-Narragansett); an existing database of chronic toxicity data compiled and largely generated by the USEPA laboratory in Duluth; chronic toxicity data submitted to USEPA under FIFRA pesticide registration requirements; and other data from the open literature (compiled in the ECOTOX data base and/or evaluated by USEPA as part of ecological risk assessments supporting pesticide registration). While the development of AquaChronTox is ongoing, priority was given to entry of data specifically for AChE inhibitors to support the analysis described in this appendix. Data coverage for AChE inhibitors within AquaChronTox is thought to be robust, though not necessarily fully comprehensive.

The AquaChronTox database contains a wide variety of extracted data, including both general experimental parameters and treatment level results for individual endpoints related to survival, growth, or reproduction. Also captured were statistically determined NOEC and LOEC values (where available) from the study authors or later re-analysis (e.g., data evaluation records completed as part of USEPA risk assessments). Both freshwater and marine/estuarine organisms were included in the data compilation. Both measured (where available) and nominal exposure concentrations are contained in the database, but measured concentrations were used for data analysis whenever available.

Initially, a total of 119 chronic studies were identified for organophosphate or carbamate insecticides. These data were then subject to additional screening wherein studies were eliminated if there were test conditions that would disqualify a test from consideration using evaluation criteria similar to that used in the development of water quality criteria (*e.g.*, unusual dilution water characteristics, use of surfactants in combination with toxicants, poor performance of control organisms). Where NOEC and LOEC values were reported for individual endpoints, a single NOEC and LOEC value for the study was established based on the most sensitive endpoint related to survival, growth, or reproduction (*i.e.*, that endpoint showing significant effects at the lowest exposure concentration). As part of this review, exposure response curves for those endpoints were evaluated qualitatively to insure that the designation of the NOEC and LOEC values was not notably ambiguous and seemed biologically reasonable. Through this review, a small number of tests were discarded because of a highly irregular exposure response curves. While it is possible that such tests represent true response profiles, they occurred in less than two percent of tests, making it seem less likely that the "true" response curve is actually non-monotonic. Another characteristic of a small number of tests was that the lowest NOEC/LOEC was reported for organism length, but there was no indication of a concomitant decrease in organism weight. In general, one expects organism weight to vary with the cube of organism length (assuming similar body morphology), so even very small changes in length would be expected to cause substantial changes in weight. In cases where small differences in length were defining the overall NOEC/LOEC without a corresponding indication of a response

in weight (or reproduction), the NOEC/LOEC for the test were increased to match the NOEC/LOEC for the next most sensitive endpoint. Finally there were a few tests for which the spacing of exposure concentrations was large (*e.g.*, greater than 3-fold concentration intervals). These tests were discarded because of the associated uncertainties in their NOEC/LOEC values.

Those tests passing the previous filters were then coded into two groups according to whether the data yielded "defined" or "unbounded" NOEC or LOEC values. In this context, the term "unbounded" means that there were either significant effects at the lowest tested concentration, or no effects at the highest tested concentration; in such cases, the NOEC and LOEC can only be expressed as inequalities. For most analyses, only those tests with defined NOEC and LOEC values were used, because of the innate difficulty in conducting distributional analyses with a mixture of defined and unbounded values. However, for some analyses unbounded values were included as check to insure that limiting analyses to defined values did not skew the analysis (*e.g.*, if eliminating tests with effects noted at the lowest tested concentration had the effect of biasing data away from tests indicating high chronic sensitivity). From the original set of 119 chronic studies, a subset of 73 tests meeting the screening criteria and with defined NOEC and LOEC values was established. For these tests, the geometric mean of the NOEC and LOEC was also calculated, denoted as "GM" in this document. GM is the equivalent of the "Maximum Acceptable Toxicant Concentration" (MATC), a term that appears frequently in the literature. MATC is not used here because its name implies a risk management judgement (what is "acceptable") that is not intrinsic to the calculated value itself.

In addition to NOEC, LOEC, and GM, regression analysis was used to estimate EC10, EC20, and EC50 concentrations for invertebrate chronic tests meeting all the previous qualification criteria. The Toxicity Response Analysis Program (TRAP, Version 1.02; U.S. EPA, Mid-Continent Ecology Division, Duluth, MN) was used to derive these values. A sigmoid model with finite tails was used to fit the exposure response data for individual endpoints, except in a very small number of cases where this model did not seem to reflect the shape of the underlying data, in which case a piecewise linear model was used. The exposure concentration was expressed on a $\log_{10}$ scale while the response variable was expressed on a linear scale. Because a log exposure scale prevents the control concentration from being entered as 0, the control exposure was assigned a concentration equal to one-tenth of the lowest exposure concentration. The test endpoint with the lowest EC20 value was identified from among the reported endpoints, and the EC10, EC20, and EC50 values for that endpoint were selected to represent that test. The EC20 was used because of its precedent as the chronic effect concentration currently used in the derivation of ALC. However, because of differences in the slope of the concentration-response curve, it was possible for an endpoint that did not have the lowest EC20 value to have the lowest EC10 or EC50 value. In the infrequent cases where this occurred, the differences were small.

Calculating ACRs also requires an acute value for the same species and chemical. Acute values were acute LC50 (or immobilization EC50) values (48-h for cladocerans; 96-h for other species) selected according to a sequence of priorities as follows:

- An LC50 provided by the study author as being paired with the chronic study
- An LC50 identified in the USEPA data evaluation record as being paired with the chronic study

- An LC50 from an acute test conducted by the same laboratory as conducted the chronic study, but potentially at a different point in time.
- An LC50 (or geometric mean of multiple LC50 values) derived from the data compilation underlying Web-ICE (Version 3.1; Raimondo et al. 2010). Values from the Web-ICE data were selected based on factors including:
    - Must meet minimum data requirements for inclusion in Web-ICE models
    - Preference given to values with measured chemistry
    - Preference given to values using flow-through methods
    - Preference given to values generated using USEPA/OPPTS methods

In the context of developing ALC, ACRs are calculated as the ratio of the acute LC50 (or EC50) to the chronic EC20 or GM. For ecological risk assessments for pesticide registration, ACRs are calculated as the ratio of the acute LC50 (or EC50) to the chronic NOEC. In this appendix, ACR is used when the endpoint is not relevant, such as when speaking conceptually, or to a point that applies to ACRs of all types. In cases where the ACR is specific to a particular chronic endpoint, subscripts are used to indicate the chronic effect measure used to calculate the ACR. For example, ACR_{GM} refers to the ratio of the LC50 to the GM, and the ACR_{NOEC} is the ratio of the LC50 to the NOEC. As defined in the introduction, an ACR_{HC5} is an ACR intended to relate the acute HC5 to the chronic HC5.

In some cases, ACR values of less than one were calculated, meaning that the LC50 was actually lower than the NOEC, GM or EC20. While it is counterintuitive that this could be the case, one must remember that the acute value is generally not derived from the same study as the chronic endpoints (because of differences in methodology), and since sources of variation exist for both the acute and chronic tests that define the ACR, it is possible for ACRs less than one to occur as a result of experimental variation. Another possible cause of ACRs less than one is where the food added during chronic testing has an effect on the bioavailability or toxicity of the chemical causing it to be less toxic than under the acute test conditions (which generally preclude feeding); this has been observed with daphnid tests and some metals. For this analysis, ACRs less than 1 were retained at their calculated value for purposes of evaluating the distribution of ACRs. In a previous analysis of ACRs, Raimondo *et al.* elected to exclude tests where ACRs were less than 1. This was not done here because of concern that it would bias the ACR distribution to higher values. This is not to suggest that ACRs less than one would be appropriate as an ACR_{HC5}, only that they should be retained during evaluation of the distribution of ACRs.

Much of the analysis that follows focuses on the ACR_{GM}, though parallel calculations were done using ACR_{NOEC} and ACR_{EC20} (for invertebrates) values. The conclusions reached using the ACR_{NOEC} are essentially the same as those obtained from the ACR_{GM}; this is because data were only used from tests with similar and relatively close spacing of exposure concentrations. This makes the ratio of the ACR_{GM} to the ACR_{NOEC} fairly consistent; for the 73 chronic tests evaluated, the median ratio of the ACR_{GM} to the ACR_{NOEC} was 1.40, with 10^{th} and 90^{th} percentiles of 1.28 and 1.55, and minimum and maximum of 1.11 and 2.00. ACR_{EC20} values were only calculated for the 26 chronic tests conducted with invertebrates. Within those data, the median ratio of the ACR_{EC20} to the ACR_{NOEC} was 1.41, the 10^{th} and 90^{th} percentiles were

1.07 and 3.11 and the minimum and maximum were 0.89 and 6.91. Comparing the ACR_{EC20} to the ACR_{GM} gave a median ratio of 1.01, the 10^{th} and 90^{th} percentiles were 0.75 and 2.41, and the minimum and maximum were 0.63 and 5.12.

Few tests of statistical significance were used to compare groups of ACRs. This is because data availability was highly irregular across species and chemicals, so data sets that compare, for example, two taxa across the same set of chemicals, are generally limited. Because of the high variability in ACRs, statistical comparisons of ACRs with low sample size lack power. Accordingly, it was decided that direct inspection of the data was sufficient for most evaluations presented.

3.0 RESULTS AND DISCUSSION

Tables 1, 2, and 3 provide a summary of the ACR_{GM}, ACR_{NOEC}, and ACR_{EC20} (respectively) data for specific combinations of species and chemicals. Figure 1 shows the cumulative distribution of ACR_{GM} values across all taxa and AChE inhibitors, showing that overall ACRs varied widely, spanning more that four orders of magnitude. Figure 2 separates the distributions for invertebrates and fish and shows that while ACRs for both groups vary considerably, the distribution of invertebrate ACRs was consistently lower, and somewhat less variable than that for fish.

Figure 3, shows the same distribution as Figure 2, except that data are coded as to whether they represent OP or carbamate chemicals. While there are fewer data for carbamate chemicals, their ACRs show a fairly wide dispersion across the overall distributions, giving little reason to believe that combining data for OP and carbamate chemicals is inappropriate.
Figure 4, shows the same distribution with data coded as to whether the test species was a freshwater or marine/estuarine organism. For the fish data, the overlap of freshwater and saltwater species is fairly broad. For invertebrates, however, 8 of the 9 saltwater ACRs lie at or above the 50^{th} percentile. Figure 5 shows the distributions of invertebrate ACRs by species; as indicated in the figure, mysid shrimp (*Americamysis bahia*) is the only marine invertebrate organism, and freshwater data are all for cladocerans, predominately *Daphnia magna*. To provide a more focused evaluation of the comparative sensitivity of mysids and *D. magna*, a subset of the overall data was extracted containing data for only those chemicals that have data for both species (Table 4). The ratio of the mysid ACR_{GM} to the daphnid ACR_{GM} was calculated for each chemical, then the logs of those ratios were evaluated with a t-test to determine if the ratios were significantly different (greater) than 1 (which would indicate that mysids were significantly more sensitive). In 6 of 8 direct comparisons, the mysid ACR_{GM} was higher than the daphnid ACR_{GM}, with an average ratio of 1.8. However, a one-tailed test ($p=0.05$) of whether this was significantly different from 1 was close to, but not quite, significant ($p=0.057$).

Some evidence that runs counter to a presumption that mysids are more sensitive than daphnids lies in three mysid chronic tests reported by Thursby et al (1990a,b; 1991). These three tests (carbaryl, dichlorvos, and propoxur) were excluded because of reduced control survival; however, analysis of these data suggest fairly low ACRs (ACR_{GM} = 0.85, 1.21, 5.29, respectively). These values would fall in the low end of the overall ACR_{GM} distribution for invertebrates, though they cannot be directly compared because ACR_{GM} values for *D. magna* are not available for these same chemicals.

While fish showed high ACRs for AChE inhibitors, this does not translate to high chronic sensitivity of fish relative to invertebrates. Because the ACR_{GM} is a ratio of LC50/GM, high ACRs can be produced not only by having low chronic effect concentrations, but also by having very high LC50 values. Relative to invertebrates, the high ACRs for fish exposed to AChE inhibitors appears to be driven largely by a very low acute sensitivity (high LC50 values), rather than by having high chronic sensitivity. This low level of acute sensitivity is affirmed by SSD analyses for AChE inhibitors conducted by Erickson (see Appendix D).

To help put the chronic sensitivity of fish in perspective, the NOECs from chronic fish tests were plotted against the acute LC50 for *D. magna* for the same chemical (Figure 6). In addition to tests with defined NOEC values, this plot also includes tests with unbounded NOECs, and values are coded with respect to whether they were derived from ELS tests or life-cycle (or partial life-cycle) tests. As points of reference, three lines were drawn representing:

- the *D. magna* LC50
- an estimate of a median acute HC5, estimated by dividing the *D. magna* LC50 by 2.89, the median acute AChE inhibitor EF derived by Erickson (Appendix D) for data sets including *D. magna*, rainbow trout, and bluegill sunfish;
- an estimate of the 90th percentile HC5, estimated by dividing the *D. magna* LC50 by 16, the 90th percentile acute AChE inhibitor EF derived by Erickson (Appendix D) for data sets including *D. magna*, rainbow trout, and bluegill sunfish.

Several inferences may be drawn for Figure 6. First, the vast majority of fish NOEC values are at or above the *D. magna* LC50, meaning that in most cases, the chronic sensitivity of fish to AChE inhibitors is less than the acute sensitivity of *D. magna* to the same chemicals. However even in cases where the fish NOEC was lower (more sensitive) than the *D. magna* LC50, almost all were at or above the estimates of acute HC5 values based on EFs calculated by Erickson (Appendix D). The conclusion here is that the ACR for fish may not be very important for estimating the chronic HC5 for AChE inhibitors, because of the greater sensitivity of invertebrates.

Another aspect of Figure 6 is the comparison of the general sensitivity of chronic tests with fish tested in life-cycle (including reproduction) versus early life stage (ELS) exposures. Based on inspection, there is some tendency for life-cycle tests to be toward the lower end of the scatter, suggesting they may be more sensitive indicators of chronic toxicity to fish. Using a different method of comparison, Raimondo *et al.*, (2007) found that ACRs from fish life-cycle tests were significantly higher than those for ELS tests. However, this tendency is not dramatic, as the distribution of ELS values encompasses the range of life-cycle values. In regard to estimating a chronic HC5, the same conclusion is reached from both tests types, that fish are comparatively insensitive to AChE inhibitors.

As outlined in the introduction, the 1985 Guidelines, calculation of the FACR requires ACRs (ACR_{GM} or ACR_{EC20}) for at least three species, encompassing at least one fish species, at least one invertebrate species, and at least one acutely sensitive freshwater species. Depending on the variability and distribution of the ACR_{GM} values, the FACR is determined in different ways. If the ACR_{GM} values are smaller for organisms with high acute sensitivity (and vice versa), then the FACR is determined by the ACR_{GM} for species whose acute sensitivity is near the FAV, rather than by the mean of all ACRs. The rationale for this approach is that if organisms with high acute sensitivity have smaller ACRs, then applying larger ACRs associated with acutely insensitive species would result in a chronic HC5 that was too low. For AChE inhibitors, ACR values are related to acute sensitivity, with invertebrates having both higher acute sensitivity and lower ACRs. Therefore, using the same logic, it seems appropriate to base the ACR_{HC5} for AChE inhibitors on invertebrate ACRs.

As shown in Figure 5, the invertebrate ACRs for AChE inhibitors are dominated by two species, *D. magna* and mysids. If the ACR_{HC5} is based on an invertebrate ACR, one must decide whether to segregate ACRs by species (e.g., use *D. magna* ACRs for freshwater ACR_{HC5} and mysids for marine ACR_{HC5}), or to use the joint distribution of all invertebrates. This decision hinges in part on the previously discussed issue of whether mysids do in fact have larger ACRs than do daphnids and, even if they do, might there be acutely sensitive freshwater organisms that have ACRs more similar to mysids than daphnids. Neither of these questions appear easily answered from the data in hand. Again drawing from the 1985 Guidelines, that methodology requires a minimum of three ACRs to include at least one fish species, one invertebrate species, and one acutely sensitive freshwater species. Given the species for which there are chronic data for AChE chemicals, the two ways these requirements would be met would be either: 1) *D. magna*, and two fish species, or 2) *D. magna*, mysids, and a fish. In the former case, the *D. magna* ACR_{GM} would be the FACR; in the latter case, the FACR would likely be the mean of the ACR values for *D. magna* and mysids, since both are acutely sensitive species.

Given that:

1) it is unclear whether mysids actually have higher ACR values than *D. magna*;
2) even if they do, they may represent ACRs for other relevant invertebrates; and
3) a FACR under the 1985 Guidelines would be calculated as the geometric mean of the ACRs for the two species

it seems reasonable to base an ACR_{HC5} on an ACR selected from the joint distribution of ACRs for all invertebrate species. Figure 7 shows the distribution of invertebrate ACR_{EC20}, ACR_{GM}, and ACR_{NOEC} combined across all AChE inhibitors and freshwater/saltwater species. Figure 8 directly compares EC20 and NOEC values, while Figure 9 compares EC20 and GM values. In all but one case, the NOEC was lower than the EC20, indicating that the level of effect at the NOEC was generally lower than 20%. The EC20 values showed greater similarity to the GM concentrations, with only a few GM values falling outside a range of 0.7 to 1.5 of the EC20.

Table 5 provides the 50^{th}, 80^{th}, and 90^{th} percentile values for each of the ACR calculation methods along with related values from other ACR analyses. The Host *et al.* (1995) and TenBrook et al. (2010) analyses were discussed in the introduction. The Raimondo *et al.* (2007) was a compilation of 456 ACR_{GM} values from a broad range of chemicals, the distributions of which were analyzed by a variety of sub-groupings. Interestingly, Raimondo et al. (2007) did not find a significant difference between ACRs for AChE inhibitors compared to other MOA. However, their comparisons across MOA pooled fish and invertebrate ACRs within a MOA, so the resulting distribution would have been very large (similar to Figure 1), and would not have reflected differences among taxa within an MOA, as has been described here for AChE inhibitors. Likewise, the Raimondo *et al.* comparison of fish and invertebrate ACRs did not show the pronounced differences shown in the current analysis, presumably because the Raimondo *et al.* comparison of fish v. invertebrates included all chemicals, and the large differences shown for AChE inhibitors likely doesn't exist for all chemical groups. While median ACR_{GM} values are similar between Raimondo *et al.* and the current analysis, limiting selection of the ACR_{HC5} for AChE inhibitors to invertebrates greatly reduces the ACRs in the upper ends of the distributions.

Another difference is that Raimondo *et al.* discarded all ACR_{GM} values that were less than 1 whereas these values were retained in the current analysis. As explained earlier, this has the potential to bias the ACR distribution toward higher values; the same sources of variability that create artificially low ACRs presumably create artificially high ACRs also, but these are not distinguishable from other values and are therefore left in the analysis. However, the bias created by data censoring is probably not high, because Raimondo *et al.* used similar data sources to the current analysis, and the total number of ACRs for AChE inhibitors reported by Raimondo *et al.* (78) is actually slightly higher than the number included in this analysis (73). A preliminary comparison of the invertebrate ACRs for AChE inhibitors from both analyses (data not shown) showed some differences in the low end of the distribution (which is expected because that is where values were excluded), but close agreement in the 50^{th} to 90^{th} percentiles.

The Host *et al.* (1995) and TenBrook *et al.* (2010) analyses differ from Raimondo *et al.* (2007) in that they are based on distributions of FACR values from ALC documents. Although these FACRs are based on ACR_{GM} (or ACR_{EC20}) values, they are not directly comparable to raw ACR_{GM} values, because FACRs reflect a variety of different derivations – for example, some may represent the ACR_{GM} for a single acutely sensitive species, while others may be the average of multiple ACR_{GM} values. That difference aside, because the current analysis suggests that invertebrate ACR values represent a logical basis for an ACR_{HC5} for AChE inhibitors, the invertebrate ACR_{GM} and/or ACR_{EC20} values from the current analysis are functionally parallel to the Host *et al.* FACR distribution. Invertebrate ACR_{GM} and ACR_{EC20} distributions from this analysis range from roughly equal to, to about 2-fold lower than, comparable values from the Host *et al.* (1995) and TenBrook *et al.* (2010) distributions.

When making this comparison, it is important to note that the degree of similarity between the Host *et al.* (1995) and TenBrook *et al.* (2010) FACR distributions and the proposed ACR_{HC5} values for AChE inhibitors is created in part by the previous finding that fish ACRs were not important, because the invertebrates have greater chronic sensitivity. If the fish were more chronically sensitive to AChE inhibitors, then the conclusions would likely change. As such, it is highly uncertain whether the same conclusion would be reached for another mode of action with different taxonomic sensitivity.

Because invertebrate ACRs for AChE chemicals show a substantial range, estimation of a chronic HC5 using an ACR_{HC5} will require a risk management decision as to where in the distribution the ACR_{HC5} will be selected. One important consideration is that because ACRs are ratios and both the numerator and denominator have uncertainty, variability in the ACRs from chance alone will be larger than the variability in either of the component values. In a Monte Carlo simulation, a theoretical distribution of ACR_{GM} values was created by assuming true values of 5.91 for the LC50 and 1 for the GM – this creates a "true" ACR of 5.91, which was the 50^{th} percentile for the invertebrate ACR_{GM} for AChE inhibitors. Both the LC50 and GM were assumed to vary within 2-fold, meaning that 95% of values would fall between 0.5x and 2x of the true value, with a log-normal distribution. This factor of 2 was based on a common "rule of thumb" regarding variability in toxicity test results; it is simply illustrative and is not based on any specific evaluation of test variability. This simulation gave 50^{th}, 80^{th}, and 90^{th} percentile ACR_{GM}s of 5.91 (the true mean), 11.0, and 15.6; the corresponding ACR_{GM} values from the

current analysis were 5.91, 13.6 and 18.8 (Table 5). Thus, if the true variability in LC50 and GM values were on the order of 2-fold, the majority of the observed range in ACR_{GM} values could be attributable to random variability alone, even if the "true" ACR were the same for all AChE inhibitors.

Less easily evaluated is the possibility that sources of error beyond random variability exist, causing extreme values. For example, in the current analysis the highest invertebrate ACR_{GM} is 255 for *D. magna* and disulfoton. The reported measured LC50 used to calculate this ACR is 13 ug/L. However, this value seems inconsistent with the much greater mortality response observed in the chronic test (37.5% survival at 0.64 ug/L), and is also inconsistent with a much lower (less than 1 ug/L) LC50 predicted by the ECOSAR system. While not certain, these latter observations suggest that the measured LC50 and resulting ACR may be too high. Although the calculated value of 255 was retained in the analysis, the potential for aberrantly high or low values emphasizes the potential inaccuracies that might be introduced by selecting values that are in the extreme tails of the distribution.

Another consideration in selecting an ACR from the distribution is that a chronic HC5 derived by dividing the acute HC5 by the ACR_{HC5} contains the uncertainties from both the acute HC5 estimation and the ACR distribution. If the acute HC5 derivation contains substantial conservatism (*e.g.*, an EF selected from a high percentile of the distribution) and the same is done for selecting an ACR_{HC5}, the estimated chronic HC5 may have an undesirably high aggregate conservatism.

Based on the experience of developing this analysis, suggestions for future study include the following:

1. Determine if additional screening of data to more carefully "match" (*e.g.*, same lab, same water, same time frame) the acute and chronic data would decrease variability in ACRs.
2. Consider additional data collection to evaluate specific toxicity values that are contributing to extreme values within ACR distributions.
3. Conduct similar analyses on chemicals in other MOAs to explore more fully whether consideration of MOA in developing ACR_{HC5}s provides a substantive decrease in uncertainty in deriving chronic HC5s as compared to more generalized ACR_{HC5} distributions.
4. Consider approaches that could be used to identify chemicals or chemical groups for which ACR_{HC5}s are likely to exceed default values (*e.g.*, "structural alerts" similar to Ahlers *et al.* 2006), and thereby result in overestimating the chronic HC5.

4.0 CONCLUSIONS

1) When compared across their distributions, ACRs for AChE inhibitors are much higher for fish than for invertebrates.
2) Despite having higher ACRs, fish generally have low acute sensitivity to AChE inhibitors, such that their chronic effect concentrations are typically higher than the acute effect concentrations for *D. magna* exposed to the same chemical, and almost certainly higher than an acute HC_5 for vertebrates and invertebrates combined; for this reason, estimation of fish ACR values is not important to estimating a chronic HC5 for AChE inhibitors for all species combined.
3) If the chronic HC5 for AChE inhibitors is estimated by applying an ACR_{HC5} to the acute HC5, it is logical to select the ACR_{HC5} from the distribution of invertebrate ACR values; doing so should produce a chronic HC5 for AChE inhibitors that is protective of both fish and invertebrate species.
4) The AChE inhibitor-specific distribution of invertebrate ACRs derived here is generally lower than those from literature analyses using datasets less specific to the AChE inhibition MOA. Accordingly, the same intended level of protection may be achieved with a higher chronic HC5 if the MOA-specific ACR_{HC5} is used.

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Figure 1: Distribution of ACR_{GM} values, combined across all AChE inhibitors and all species.

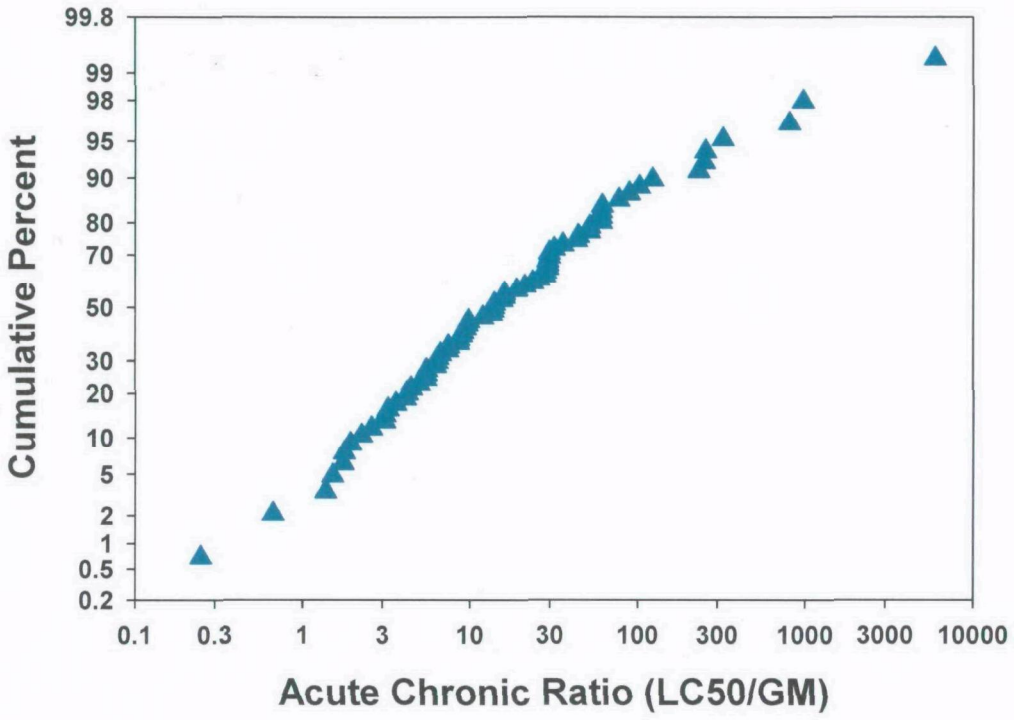

Figure 2: Distribution of fish and invertebrate ACR_{GM} values for all AChE inhibitors.

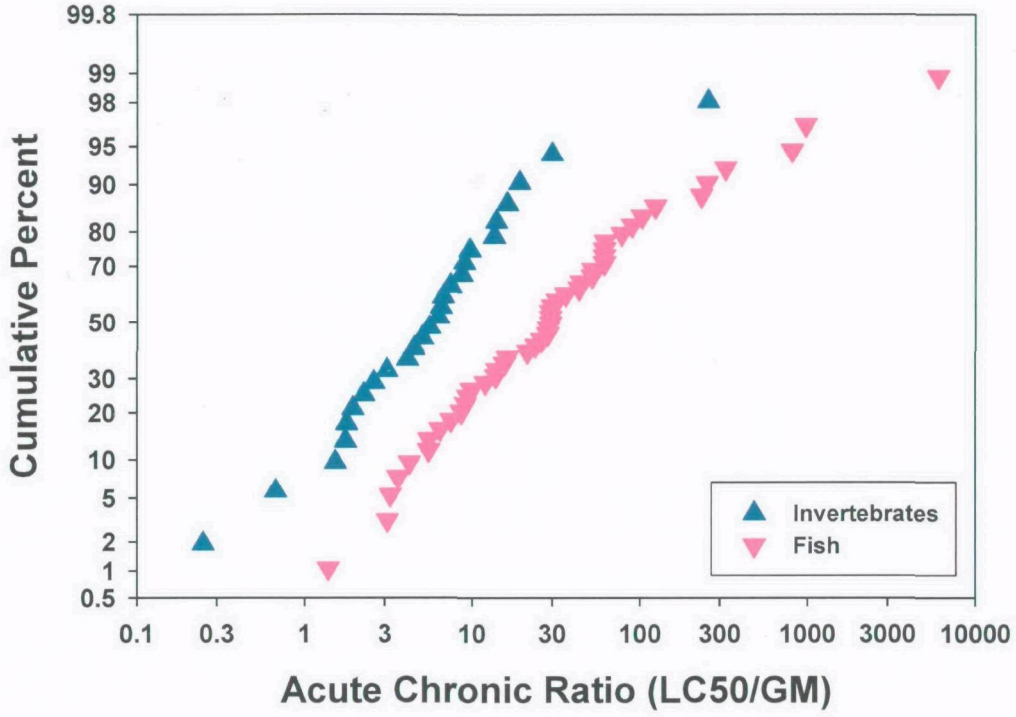

Figure 3: Distribution of fish and invertebrate ACR_{GM} values for all AChE inhibitors, coded to indicate data for carbamate and organophosphate chemicals.

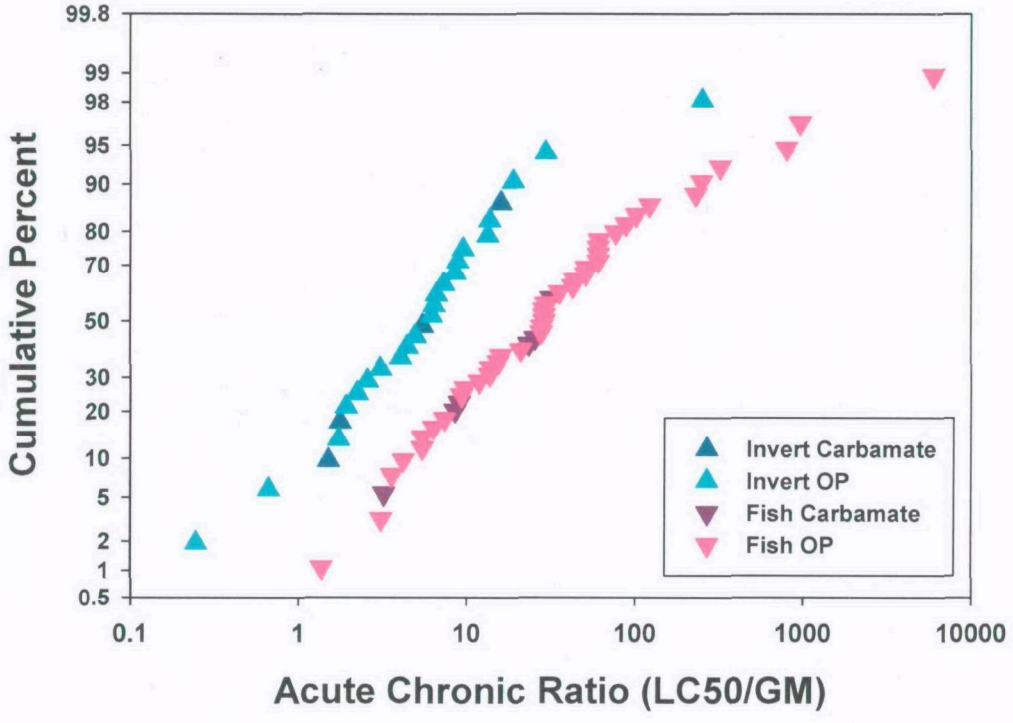

Figure 4: Distribution of fish and invertebrate ACR_{GM} values for all AChE inhibitors, coded to indicate data for freshwater and saltwater species.

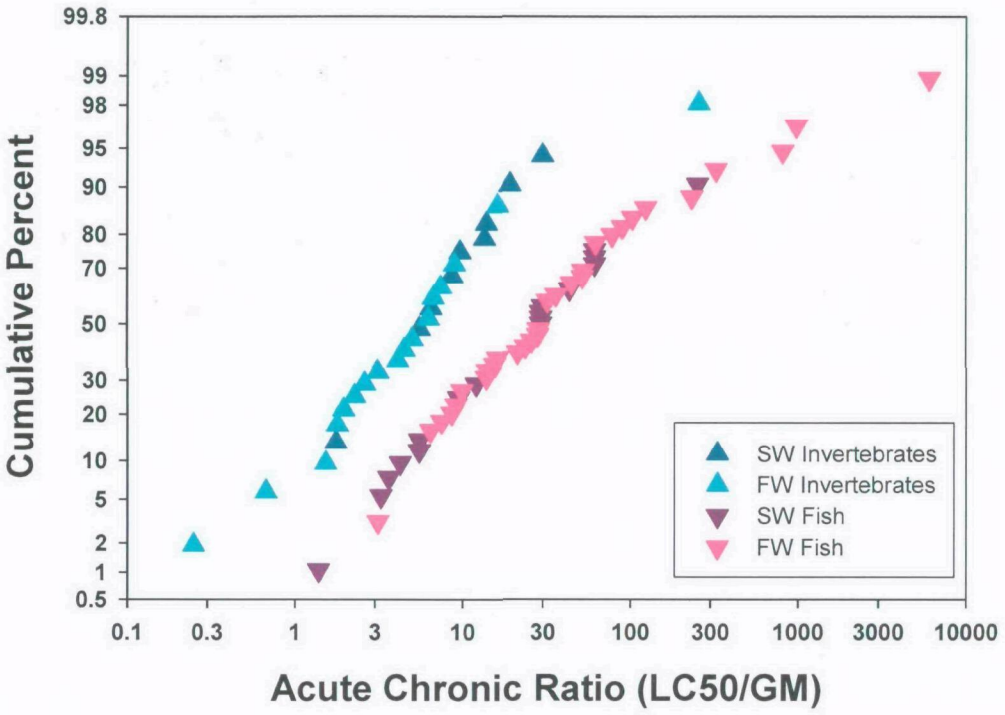

Figure 5: Distributions of ACR_{EC20} values for individual invertebrate species, combined across all AChE inhibitors.

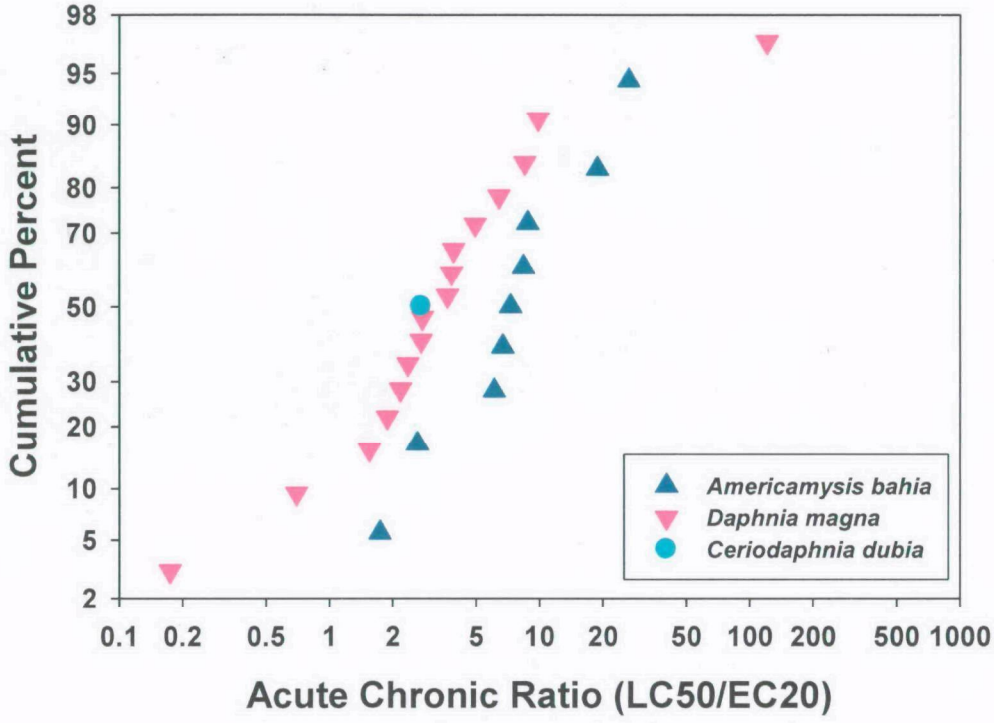

Figure 6: NOEC values from fish chronic tests with AChE inhibitors plotted as a function of the *Daphnia magna* acute LC50 and estimates of acute HC5s for the same chemical. See text for detailed explanation of the lines.

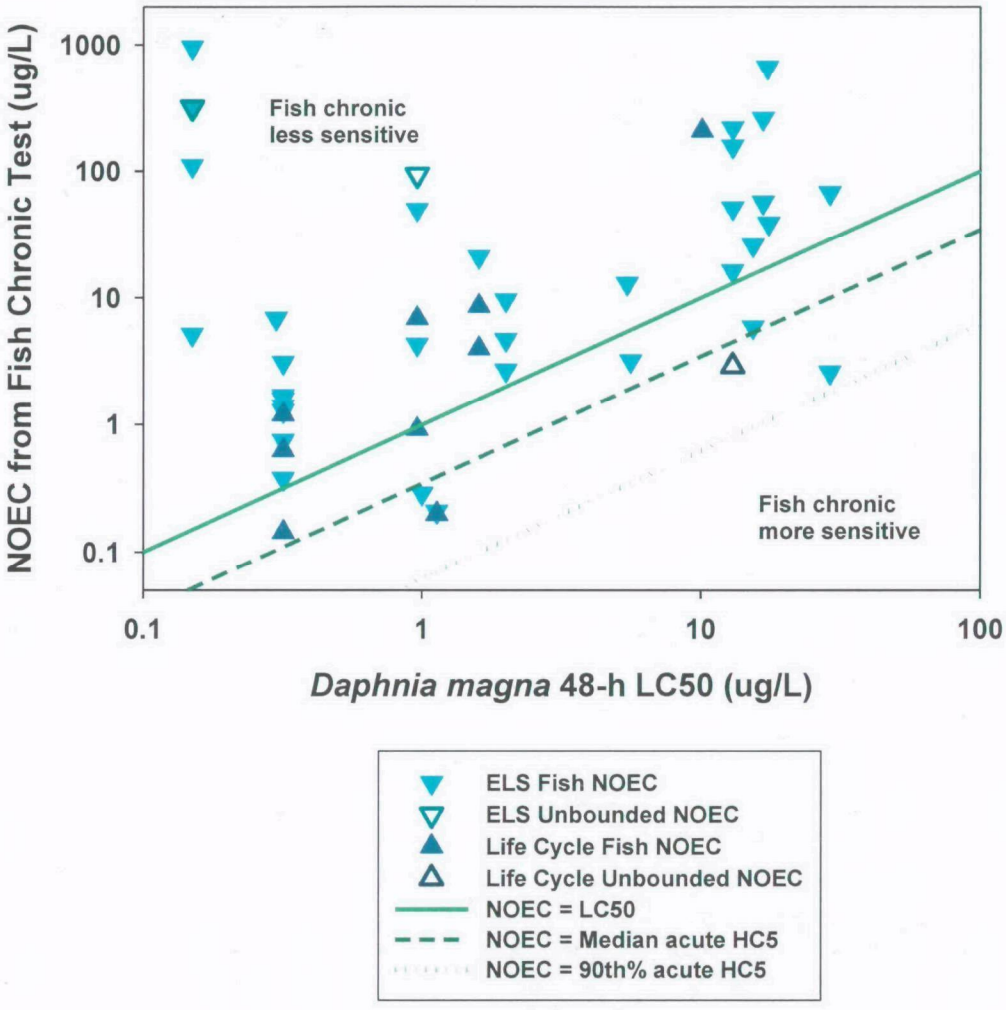

Figure 7: Distribution of ACR_{NOEC}, ACR_{GM}, and ACR_{EC20} values for invertebrates, combined across all AChE inhibitors.

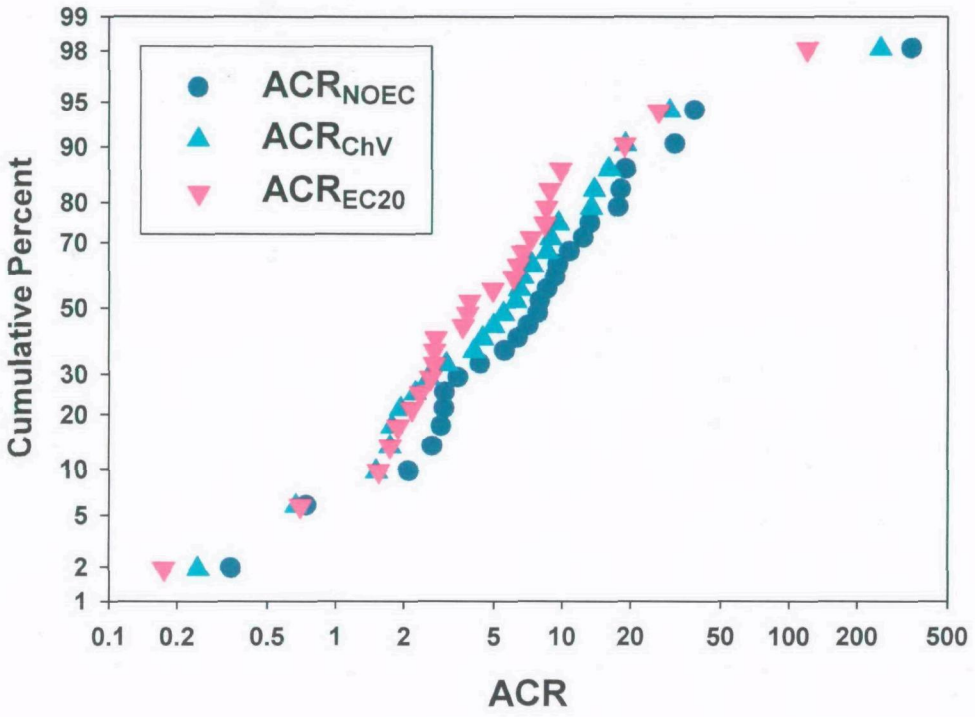

Figure 8: Comparison of EC20 and NOEC values for invertebrate species combined across all AChE inhibitors. Solid line designates unity, dashed line shows 2x deviation from unity, and dotted line shows 4x deviation from unity.

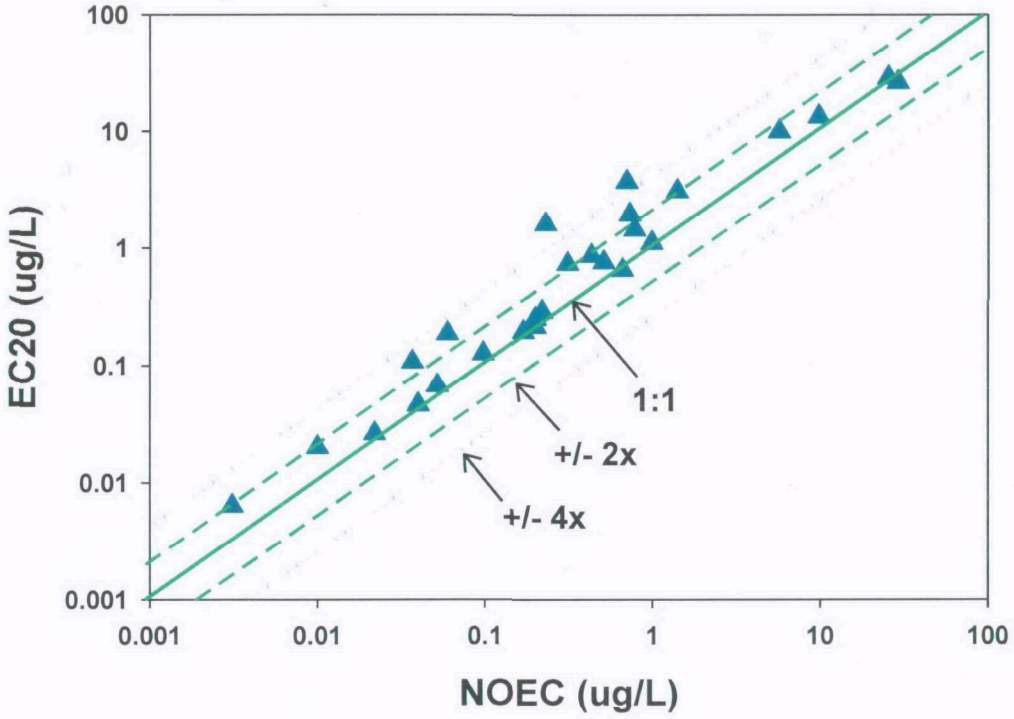

Figure 9: Comparison of EC20 and GM values for invertebrate species combined across all AChE inhibitors. Solid line designates unity, dashed line shows 2x deviation from unity, and dotted line shows 4x deviation from unity.

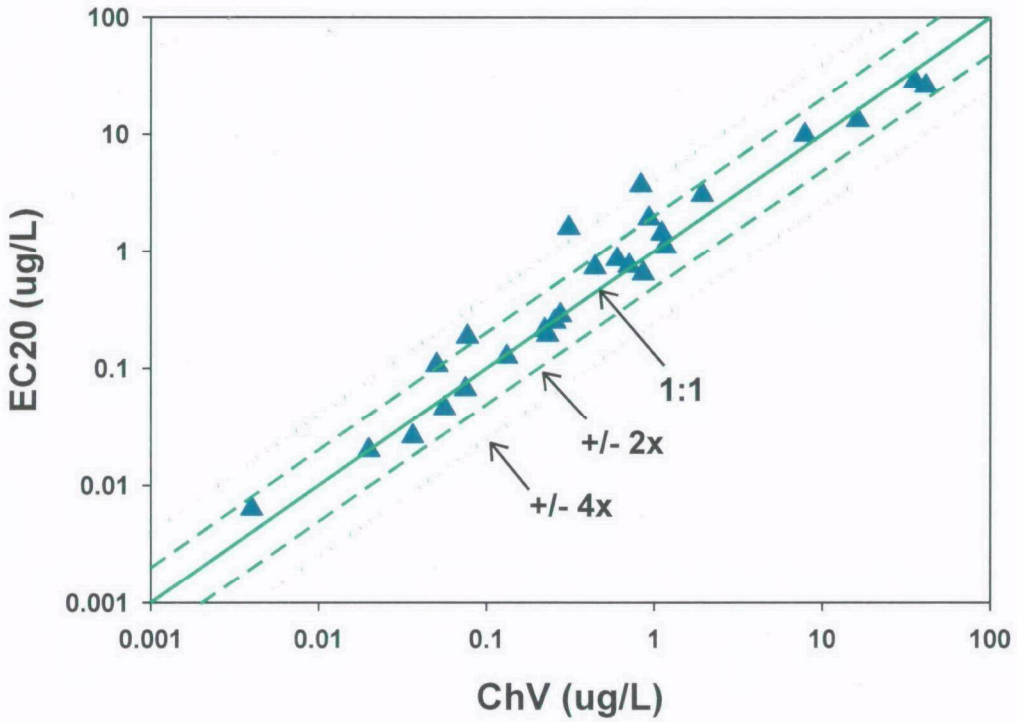

Table 1: ACR_{GM} values used in the analysis. Highlighted values represent the geometric mean of multiple values.

	Americamysis bahia	Ceriodaphnia dubia	Daphnia magna	Cyprinodon variegatus	Menidia beryllina	Menidia peninsulae	Jordanella floridae	Oncorhynchus mykiss	Opsanus beta	Pimephales promelas	Geomean Invert	Geomean Fish	Geomean all
Aminocarb										31.96		31.96	31.96
Carbaryl (Sevin)										23.82		23.82	23.82
Carbofuran			1.65							8.61	1.65	8.61	3.77
Methomyl	5.55		16.14	3.25						25.84	9.46	9.16	9.31
Propoxur (Baygon)										9.12		9.12	9.12
Azinphos-methyl				9.43				14.08				11.52	11.52
Carbophenothion										28.05		28.05	28.05
Chlorpyrifos (Dursban)	1.75		3.11	42.25	3.61	1.38			29.88	151.50	2.33	15.71	9.11
Diazinon	13.51		4.12	250.63						793.56	7.46	445.97	57.67
Dichlorvos (DDVP)				5.53				13.87		21.37		11.79	11.79
Disulfoton			255.44	43.32				9.87		28.93	255.44	23.13	42.16
Ethoprop	9.66		1.94	12.02							4.33	12.02	6.09
Fensulfothion										6.44		6.44	6.44
Fenthion								44.52				44.52	44.52
Fonofos	6.50		4.49	4.23				7.48			5.40	5.63	5.51
Malathion			6.71	5.50			36.05	3.13			6.71	8.53	8.03
Methidathion	19.11		8.15								12.48		12.48
Methyl parathion		2.59	0.41							15.62	1.03	15.62	2.55
Naled	13.89		2.26							324.37	5.60	324.37	21.67
Parathion	29.89										29.89		29.89
Phosmet			5.01					52.06			5.01	52.06	16.15
Profenofos	8.65		6.27								7.36		
Terbufos										28.38		28.38	
Geomean Carbamate	5.55		5.15	3.25						17.29	5.28	13.09	9.67
Geomean OP	10.02	2.59	4.97	16.91	3.61	1.38	36.05	13.98	29.88	49.94	6.37	20.72	12.67
Geomean All	9.38	2.59	4.99	14.08	3.61	1.38	36.05	13.98	29.88	34.19	6.21	19.11	10.52

Table 2: ACR_{NOEC} values used in the analysis. Highlighted values represent the geometric mean of multiple values.

	Americamysis bahia	Ceriodaphnia dubia	Daphnia magna	Cyprinodon variegatus	Menidia beryllina	Menidia peninsulae	Jordanella floridae	Oncorhynchus mykiss	Opsanus beta	Pimephales promelas	Geomean Invert	Geomean Fish	Geomean all
Aminocarb										50.13		50.13	50.13
Carbaryl (Sevin)										42.86		42.86	42.86
Carbofuran			2.51							12.49	2.51	12.49	5.59
Methomyl	7.90		19.29	4.46						37.02	12.35	12.85	12.60
Propoxur (Baygon)										13.23		13.23	13.23
Azinphos-methyl				13.50				17.93				15.56	15.56
Carbophenothion										37.86		37.86	37.86
Chlorpyrifos (Dursban)	3.50		4.40	60.11	5.60	1.98			48.57	212.69	3.92	23.32	14.01
Diazinon	18.26		5.65	341.86						1095.70	10.15	612.03	78.84
Dichlorvos (DDVP)				7.66				19.42		28.09		16.10	16.10
Disulfoton			351.35	61.73				13.64		44.08	351.35	33.35	60.09
Ethoprop	13.41		2.70	16.41							6.02	16.41	8.41
Fensulfothion										8.94		8.94	8.94
Fenthion								64.15				64.15	64.15
Fonofos	9.39		6.45	5.93				10.64			7.78	7.94	7.86
Malathion			8.67	8.25			40.58	4.52			8.67	11.48	10.70
Methidathion	31.82		11.03								18.73		18.73
Methyl parathion		3.05	0.51							17.29	1.25	17.29	3.00
Naled	17.81		3.06							478.26	7.38	478.26	29.65
Parathion	38.71										38.71		38.71
Phosmet			7.18					71.88			7.18	71.88	22.72
Profenofos	10.91		8.05								9.37		
Terbufos										38.19		38.19	
Geomean Carbamate	7.90		6.95	4.46						26.53	7.26	19.71	14.13
Geomean OP	14.47	3.05	6.75	23.86	5.60	1.98	40.58	19.45	48.57	67.94	8.80	28.83	17.58
Geomean All	13.53	3.05	6.78	19.81	5.60	1.98	40.58	19.45	48.57	48.56	8.58	26.96	14.69

Table 3: ACR_{EC20} values used in the analysis. Highlighted values represent the geometric mean of multiple values.

	Americamysis bahia	Ceriodaphnia dubia	Daphnia magna	Geomean
Aminocarb				
Carbaryl (Sevin)				
Carbofuran			2.04	2.04
Methomyl	8.84		3.67	5.69
Propoxur (Baygon)				
Azinphos-methyl				
Carbophenothion				
Chlorpyrifos (Dursban)	1.75		3.84	2.59
Diazinon	2.64		4.97	3.62
Dichlorvos (DDVP)				
Disulfoton			121.12	121.12
Ethoprop	6.13		1.56	3.09
Fensulfothion				
Fenthion				
Fonofos	7.33		2.75	4.49
Malathion			2.79	2.79
Methidathion	26.71		9.19	15.66
Methyl parathion		2.75	0.35	0.98
Naled	6.73		2.38	4.00
Parathion	18.92			18.92
Phosmet			3.92	3.92
Profenofos	8.42		6.45	7.37
Terbufos				
Geomean Carbamate	8.84		2.73	4.04
Geomean OP	7.03	2.75	4.09	4.98
Geomean All	7.21	2.75	3.84	4.84

Table 4: ACR_{GM} values for AChE inhibitors with defined values for both *Daphnia magna* and mysids.

Chemical	Mysid ACR	D. magna ACR	Mysid/magna	Log mysid/magna
Chlorpyrifos (Dursban)	1.75	3.84	0.456	-0.341
Diazinon	2.64	4.97	0.532	-0.274
Ethoprop	6.13	1.56	3.941	0.596
Fonofos	7.33	2.75	2.664	0.426
Methidathion	26.71	9.19	2.907	0.463
Methomyl	8.84	3.67	2.411	0.382
Naled	6.73	2.38	2.832	0.452
Profenofos	8.42	6.45	1.306	0.116
			mean =	0.228
1-tailed t p=0.05 df=7	1.895		SD =	0.357
2-tailed t p=0.05 df=7	2.365		t =	1.803

Not significantly different under 1-tailed test at p=0.05
Not significantly different under 2-tailed test at p=0.05

Table 5: Distributional values for ACRs from the current analysis and literature sources.

ACR Distribution Source/Type	Percentile		
	50th	**80th**	**90th**
Present Analysis:			
AChE inhibitors, fish only ACR_{NOEC}	42.9	118	334
AChE inhibitors, fish only ACR_{GM}	29.2	79.0	247
AChE inhibitors, invertebrates only ACR_{NOEC}	7.98	17.9	30.6
AChE inhibitors, invertebrates only ACR_{GM}	5.91	13.6	18.8
AChE inhibitors, invertebrates only ACR_{EC20}	3.88	8.63	18.0
Raimondo *et al.* (2007):			
Combined chemicals, all species ACR_{GM}	8.3	NR	79.5
AChE inhibitors, all species ACR_{GM}	6.4	NR	60.2
Combined chemicals, fish only ACR_{GM}	9.3	NR	90.0
Combined chemicals, invertebrates only ACR_{GM}	7.5	NR	68.3
Host *et al.* (1995):			
Combined chemicals, freshwater FACR	7.1	17.9	29.1
Combined chemicals, saltwater FACR	4.4	13.5	24.2
TenBrook *et al.* (2010)			
Combined chemicals, freshwater FACR	NR	12.4	NR

NR = Not Reported