

Version 1.2

Adding Error Estimates to EPA's NH_3 Recalculation Criteria

Addendum to Richards 2016 Draft Report.

Version 1.2

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Justification

EPA reported Final Acute Values (FAVs) and chronic values as point estimates without any error associated in their ammonia water criteria (USEPA 2013b). Richards 2016 discussed that including error estimates was crucial for useful ammonia criteria and that omission of error estimates was cause for concern. Subsequently error estimates were made using EPA's sensitivity values reported by EPA in Appendix A (USEPA 2013b) in this addendum.

Methods

Species toxicity values were compiled from Appendix A (USEPA 2013b). Geometric means and standard errors and 90% confidence intervals (CIs) of the means were calculated, as was the sample size. One standard error and 90% CIs as opposed to more commonly used two standard errors and 95% CIs were calculated to avoid the extremely large variability estimates associated with the dataset and which would have resulted in essentially useless wide intervals. Discrepancies between calculated values presented here and by EPA for several taxa are noted and discussed. Final Acute Values (FAVs) were then calculated at one standard error less than the mean and one standard error greater than the mean. Chronic values were calculated by using results from the error estimates for FAVs.

Results

Geometric means, standard errors, 90% CIs, and sample sizes (N) are in Table 1. It was evident that there was a wide range in error estimates and that a simple geometric mean was not likely to reflect sensitivity values with any precision. Many species had been only tested on one occasion (N = 1) and no error estimates were obtainable. 90% CIs for species with few tests (N < 5 or so) had very large intervals. For example, *Hyaella azteca* (N = 3 test scores) 90% CIs were between 53.40 and 694.44 mg TAN/L (Table 1). cursory examination showed a trend that increased sample size resulted in less error estimation, whereas fewer test samples tended to have wider error estimates.

Table 1. Species Mean Acute Values (mg TAN/L), +/- 1 standard error (S.E.), lower and upper 90% confidence intervals (CIs), and number of tests (N) used in EPA 2013b, Appendix A.

| Species | SMAV (mg TAN/L) | - 1 S.E. | + 1S.E. | Lower 90% CI | Upper 90% CI | N |
|--------------------------------|--------------------|----------|---------|-----------------|-----------------|---|
| <i>Acipenser brevirostrum</i> | 156.7 | NA | NA | NA | NA | 1 |
| <i>Actinonaias ligamentina</i> | 63.89 | 52.96 | 74.82 | 42.71 | 95.55 | 4 |
| <i>Actinonaias pectorosa</i> | 79.46 | 75.71 | 79.46 | 58.99 | 107.03 | 2 |
| <i>Alasmidonta heterodon</i> | >109.0 | NA | NA | NA | NA | 1 |
| <i>Asellus aquaticus</i> | 378.2 | 333.99 | 422.41 | 304.31 | 470.03 | 9 |
| <i>Caecidotea racovitzai</i> | 387 | 313.50 | 460.50 | 222.26 | 673.86 | 3 |
| <i>Callibaetis skokianus</i> | 364.6 | 302.16 | 427.04 | 123.64 | 1075.08 | 2 |
| <i>Callibaetis</i> sp. | 166.7 | NA | NA | NA | NA | 1 |

| | | | | | | |
|---------------------------------------|---------|---------|---------|---------|---------|----|
| <i>Campostoma anomalum</i> | 115.9 | NA | NA | NA | NA | 1 |
| <i>Catostomus commersonii</i> | 157.5 | 123.27 | 191.73 | 105.16 | 235.97 | 9 |
| <i>Catostomus platyrhynchus</i> | 136.2 | 124.14 | 148.26 | 105.16 | 176.39 | 3 |
| <i>Ceriodaphnia acanthina</i> | 154.3 | NA | NA | NA | NA | 1 |
| <i>Ceriodaphnia dubia</i> | 134.2 | 114.03 | 154.37 | 102.81 | 175.09 | 14 |
| <i>Chasmistes brevirostris</i> | 69.36 | 45.32 | 93.40 | 7.77 | 618.79 | 2 |
| <i>Chironomus riparius</i> | 1029 | NA | NA | NA | NA | 1 |
| <i>Chironomus tentans</i> | 451.8 | 340.17 | 563.43 | 361.84 | 824.53 | 6 |
| <i>Chydorus sphaericus</i> | 162.6 | NA | NA | NA | NA | 1 |
| <i>Cottus bairdii</i> | 222.2 | NA | NA | NA | NA | 1 |
| <i>Crangonyx pseudogracilis</i> | 270.5 | 201.87 | 339.13 | 97.75 | 397.61 | 6 |
| <i>Crangonyx</i> sp. | 122.2 | 78.08 | 166.32 | 12.49 | 1194.65 | 2 |
| <i>Cyprinella lutrensis</i> | 196.1 | 186.30 | 205.90 | 143.01 | 268.77 | 2 |
| <i>Cyprinella spiloptera</i> | 83.8 | 77.48 | 90.12 | 67.23 | 104.46 | 3 |
| <i>Cyprinella whipplei</i> | 80.94 | NA | NA | NA | NA | 1 |
| <i>Cyprinus carpio</i> | 106.3 | 84.64 | 127.96 | 58.60 | 192.68 | 3 |
| <i>Daphnia magna</i> | 157.7 | 127.09 | 188.31 | 111.57 | 222.86 | 13 |
| <i>Daphnia pulicaria</i> | 99.03 | NA | NA | NA | NA | 1 |
| <i>Deltistes luxatus</i> | 56.62 | 42.88 | 70.36 | 12.23 | 262.17 | 2 |
| <i>Dendrocoelum lacteum</i> | 119.5 | NA | NA | NA | NA | 1 |
| <i>Drunella grandis</i> | 442.4 | 389.96 | 494.84 | 312.94 | 625.36 | 3 |
| <i>Enallagma</i> sp. | 164 | NA | NA | NA | NA | 1 |
| <i>Epioblasma capsaeformis</i> | 31.14 | 21.23 | 41.05 | 12.29 | 78.88 | 3 |
| <i>Erythromma najas</i> | 2515 | 1824.38 | 3205.62 | 1127.79 | 5607.37 | 3 |
| <i>Etheostoma nigrum</i> | 71.45 | 61.64 | 81.26 | 54.17 | 94.23 | 6 |
| <i>Etheostoma spectabile</i> | 77.17 | 70.87 | 83.47 | 46.08 | 129.25 | 2 |
| <i>Fluminicola</i> sp. ² | > 62.15 | NA | NA | NA | NA | 1 |
| <i>Fusconaia masoni</i> | 47.4 | NA | NA | NA | NA | 1 |
| <i>Gambusia affinis</i> | 219.3 | 182.33 | 256.27 | 147.53 | 326.12 | 4 |
| <i>Gasterosteus aculeatus</i> | 281.5 | 225.97 | 337.03 | 191.86 | 413.00 | 7 |
| <i>Hyalella azteca</i> | 192.6 | 108.01 | 277.19 | 53.40 | 694.44 | 3 |
| <i>Hybognathus amarus</i> | 72.55 | NA | NA | NA | NA | 1 |
| <i>Ictalurus punctatus</i> | 142.4 | 133.06 | 151.74 | 130.15 | 162.08 | 27 |
| <i>Lampsilis abrupta</i> | 19.57 | 13.99 | 25.15 | 3.23 | 118.58 | 2 |
| <i>Lampsilis cardium</i> ¹ | 89.79 | 59.84 | 119.74 | 40.96 | 196.86 | 4 |
| <i>Lampsilis fasciola</i> | 48.11 | 37.38 | 58.84 | 28.47 | 81.33 | 4 |
| <i>Lampsilis higginsii</i> | 41.9 | 38.31 | 45.49 | 24.41 | 71.92 | 2 |
| <i>Lampsilis rafinesqueana</i> | 69.97 | 61.40 | 69.97 | 48.85 | 99.99 | 3 |

| | | | | | | |
|---|--------------|---------|---------|--------|---------|-----|
| <i>Lampsilis siliquoidea</i> ¹ | 49.75 | 41.62 | 41.62 | 37.40 | 66.18 | 17 |
| <i>Lasmigona subviridis</i> | 23.41 | 22.60 | 24.22 | 21.15 | 25.91 | 3 |
| <i>Lepomis cyanellus</i> | 150.8 | 136.02 | 165.58 | 110.20 | 180.46 | 4 |
| <i>Lepomis gibbosus</i> | 77.53 | 48.11 | 106.95 | 31.75 | 189.34 | 4 |
| <i>Lepomis macrochirus</i> | 104.5 | 97.03 | 111.97 | 87.04 | 112.46 | 31 |
| <i>Limnodrilus hoffmeisteri</i> | 170.2 | NA | NA | NA | NA | 1 |
| <i>Lumbriculus variegatus</i> | 218.7 | 148.51 | 288.89 | 123.07 | 412.79 | 7 |
| <i>Lymnaea stagnalis</i> | 88.62 | NA | NA | NA | NA | 1 |
| <i>Micropterus dolomieu</i> | 150.6 | 119.86 | 181.34 | 93.19 | 243.50 | 4 |
| <i>Micropterus salmoides</i> | 86.02 | 81.44 | 90.60 | 61.47 | 120.37 | 2 |
| <i>Micropterus treculii</i> | 54.52 | NA | NA | NA | NA | 1 |
| <i>Morone chrysops</i> | 144 | NA | NA | NA | NA | 1 |
| <i>Morone saxatilis</i> | 246.2 | 203.05 | 289.35 | 170.21 | 329.50 | 11 |
| <i>Morone saxatilis x chrysops</i> | 70.22 | 65.21 | 75.23 | 61.78 | 79.81 | 12 |
| <i>Musculium transversum</i> | 89.36 | 78.53 | 100.19 | 62.72 | 127.30 | 3 |
| <i>Notemigonus crysoleucas</i> ¹ | 114 | 79.99 | 148.01 | 47.71 | 272.42 | 3 |
| <i>Notropis topeka</i> | 96.72 | 75.32 | 118.12 | 50.69 | 184.54 | 3 |
| <i>Oncorhynchus aguabonita</i> | 112.1 | NA | NA | NA | NA | 1 |
| <i>Oncorhynchus clarkii</i> | 78.92 | 67.53 | 90.31 | 60.04 | 103.73 | 8 |
| <i>Oncorhynchus gorbuscha</i> | 180.7 | 163.87 | 197.53 | 100.35 | 325.27 | 2 |
| <i>Oncorhynchus kisutch</i> | 87.05 | 81.98 | 92.12 | 77.95 | 97.20 | 8 |
| <i>Oncorhynchus mykiss</i> ¹ | 91.64 | 88.49 | 94.79 | 86.58 | 97.00 | 149 |
| <i>Oncorhynchus mykiss</i> ² | 82.88 | 79.95 | 85.81 | 78.17 | 87.89 | 118 |
| <i>Oncorhynchus tshawytscha</i> | 82.39 | 72.51 | 92.27 | 62.14 | 109.24 | 4 |
| <i>Orconectes immunis</i> | 1550 | 1355.51 | 1744.49 | 701.71 | 3422.79 | 2 |
| <i>Orconectes nais</i> | 303.8 | NA | NA | NA | NA | 1 |
| <i>Oreochromis mossambicus</i> | 185.2 | NA | NA | NA | NA | 1 |
| <i>Pachydiplax longipennis</i> | 233 | 159.67 | 306.33 | 31.95 | 1699.59 | 2 |
| <i>Philarctus quaeris</i> | 994.5 | 957.71 | 1031.29 | 787.36 | 1256.18 | 2 |
| <i>Physa gyrina</i> | 164.5 | 154.47 | 174.53 | 145.44 | 185.98 | 6 |
| <i>Pimephales promelas</i> | 159.2 | 150.10 | 168.30 | 125.75 | 156.15 | 73 |
| <i>Planorbella</i> | 211.6 | 200.46 | 222.74 | 151.70 | 295.04 | 2 |
| <i>Pleurocera uncialis</i> | 68.54 | NA | NA | NA | NA | 1 |
| <i>Poecilia reticulata</i> | 74.66 | 26.31 | 123.01 | 16.27 | 342.74 | 4 |
| <i>Potamilus ohioensis</i> | >109.0 | NA | NA | NA | NA | 1 |
| <i>Procambarus clarkii</i> | 138 | 109.16 | 166.84 | 36.90 | 516.29 | 4 |
| <i>Prosopium williamsoni</i> | 51.93 | 34.81 | 69.05 | 19.83 | 135.98 | 3 |

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|------------------------------------|--------|--------|--------|--------|--------|----|
| <i>Pseudacris crucifer</i> | 61.18 | 47.65 | 74.71 | 15.14 | 247.29 | 2 |
| <i>Pseudacris regilla</i> | 83.71 | 55.88 | 111.54 | 41.20 | 170.07 | 5 |
| <i>Pyganodon grandis</i> | 70.73 | 69.00 | 72.46 | 60.59 | 82.58 | 2 |
| <i>Rana pipiens</i> | 96.38 | 65.13 | 127.63 | 12.44 | 746.73 | 2 |
| <i>Salmo salar</i> | 183.3 | 162.17 | 204.43 | 150.13 | 223.70 | 20 |
| <i>Salmo trutta</i> | 101.99 | 98.71 | 105.27 | 92.84 | 112.05 | 3 |
| <i>Salvelinus fontinalis</i> | 156.3 | 149.55 | 163.05 | 119.01 | 205.28 | 2 |
| <i>Salvelinus namaycush</i> | 159.3 | 149.81 | 168.79 | 138.50 | 183.32 | 4 |
| <i>Sander vitreus</i> | 117.1 | 86.90 | 147.30 | 61.57 | 197.76 | 5 |
| <i>Simocephalus vetulus</i> | 142.9 | 126.57 | 159.23 | 109.22 | 187.00 | 4 |
| <i>Skwala americana</i> | 192.4 | 186.60 | 198.20 | 159.07 | 232.74 | 2 |
| <i>Stenelmis sexlineata</i> | 735.9 | NA | NA | NA | NA | 1 |
| <i>Tubifex tubifex</i> | 216.5 | NA | NA | NA | NA | 1 |
| <i>Utterbackia imbecillis</i> | 46.93 | 39.90 | 53.96 | 35.52 | 62.00 | 9 |
| <i>Venustaconcha ellipsiformis</i> | 23.12 | NA | NA | NA | NA | 1 |
| <i>Villosa iris</i> | 34.23 | 26.41 | 42.05 | 22.52 | 52.03 | 10 |
| <i>Xenopus laevis</i> | 122.5 | 88.55 | 156.45 | 72.42 | 204.44 | 9 |

¹Geometric means calculated in this table were different than EPA (2013b) values

²Using EPA 'bolded' test values (USEPA 2013b, Appendix A).

Golden shiner (*Notemigonus crysoleucas*)

There were three tested values reported by EPA for Golden shiner (*Notemigonus crysoleucas*): 162.2, 144.6, and 63.02 mg TAN/L (EPA 2013b, Appendix A. page 124). However, EPA reported the lowest value, 63.02 as the final SMAV not the geometric mean of 114 (Table 1, this document). The only explanation I can determine for EPA's use of lowest value and not geometric mean is from the Guidelines Deriving Numerical Document, Page 15, Section IV. G.

“If the available data indicate that one or more life stages are at least a factor of two more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the Species Mean Acute Value because a species can only be considered protected from acute toxicity if all life stages are protected.”

However, in EPA (2013b) page 21, EPA states that, “Data that were suitable for the derivation of a freshwater FAV are presented in Appendix A.”, including all three Golden shiner toxicity values. Alternatively, in Appendix A, page 124 (EPA2013b), the lowest value of 63.02 is also accompanied by a value of 8.7 g which apparently represents the weight of the tested shiner. There is no statement regarding whether this test was on a different life stage (e.g. egg, larvae, or juvenile) than the other two entries. Juvenile Golden shiners appear to weigh much less than 8.7 g (Hickman and Kilambi 1974, Melandri date unknown, Pearson et al. 2012) so it seems likely a weight of 8.7 g is not an early life stage. Contrarily, the mean standard weight of 8.3 g is

typically used for stocking (Pearson et al. 2012). Therefore, the geometric mean of 114 should have been used for the SMAV and not the lowest value of 63.02 for EPA's ammonia criteria.

The Golden shiner was one of the four most sensitive genera used in the Richards (2016) recalculation process for Mill Creek using the questionable 63.02 SMAV (mg TAN/L). Further investigation is critical to determine EPA's rationale for use of lowest test value of 63.02 instead of the geometric mean of 114 derived from the three test values that EPA (2013b) suggested as 'suitable'.

Fluminicola sp. (Pebblesnail)

The pebblesnail, *Fluminicola* sp. had the lowest GMAV, >62.15 mg TAN/L in the Richards 2016 recalculation report and EPA 2013b document. The use of the 62.15 values is questionable. In the EPA guidelines document IV. Final Acute Value, E. 5, page 15 EPA states,

"If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species."

This rationale for inclusion of a species reported with "greater than" values was actually reversed of the above EPA quote for *Fluminicola* sp. because the **inclusion** not rejection of this taxon unnecessarily lowered the Final Acute Value by including acute values for a **sensitive** species not a **resistant** species.

In addition, as reported in Richards 2016 recalculation, *Fluminicola* sp. likely doesn't occur in the Mill Creek site and was used as a surrogate for the highly invasive, ecosystem altering New Zealand mudsnail, *P. antipodarum*, which does occur in the Mill Creek site.

Lampsilis cardium (Plain pocketbook mussel)

EPA (2013b) used and SMAV of 50.51 mg TAN/L for *Lampsilis cardium* based on four toxicity test results (Appendix A., page 125 and 126). It appears EPA elected to use the two lowest of the four values to calculate the geometric mean possibly because the two lowest values were from tests on 1-2 day old juveniles as opposed to 3-5 day old juveniles. However, all four tested values were juveniles and were not different life stages even though there was > 2 times differences. Therefore, the geometric mean of 89.79 should have been used on all four values. In many instances in EPA 2013b Appendix A, within species differences can exceed two times the lowest and highest values and EPA chooses to include all these values in calculation of the SMAV. These EPA discrepancies need to be investigated.

Lampsilis siliquoidea (Fatmucket mussel)

Similarly to *Lampsilis cardium*, EPA excluded several test values that were included in Appendix A for calculating *L. siliquoidea* SMAVs. These exclusions were apparently because,

“^d The EC50s reported in this study were based on nominal concentrations. Percent nominal concentrations of measured ammonia concentrations on exposure days 0 and 4 declined from 104 to 63. EC50s based on measured concentrations were estimated from the reported EC50s based on nominal concentrations by multiplying by 0.835 or the average of the percent nominal concentrations of measured concentrations from ammonia measurements made on exposure days 0 and 4 in the study.” (USEPA 2013b, Appendix A).

EPA calculated an SMAV for *L. siliquoidea* of 55.42 mg TAN/L by omitting several values, whereas, the inclusion of all the tested values resulted in an SMAV of 49.75 mg TAN/L.

Oncorhynchus mykiss (Rainbow trout)

EPAs final SMAV for rainbow trout was 82.88 mg TAN/L (EPA 2013b, Appendix A., page 120) after their deletion of several test values. Inclusion of these values resulted in an SMAV of 91.64 mg TAN/L. Error estimates for both estimates (EPAs and this addendums) are in Table 1.

Final Acute Values based on Error

Final Acute Values (FAVs) were calculated using error estimates from Table 1 for the four most sensitive taxa, *Fluminicola*, *Notemigonus*, *Pseudacris*, and *Hybognathus*. A FAV for the mean minus one standard deviation was made (Table 2) as was a FAV for the mean plus one standard deviation (Table 3). *Fluminicola* and *Hybognathus* GMAVs had only one test value therefore error estimates were simply calculated as +/- 10% of the mean for *Hybognathus* and the low error estimate for *Fluminicola* was kept as the GMAV reported by EPA because it was reported as > 62.15. The high error estimate for *Fluminicola* was +10% of the GMAV. Error estimated FAVs were 66.13 for low estimate and 88.79 for the high estimate.

Table 2

-1 S.E. (-10% mean for GMAVs with only one test value, i.e. *Fluminicola* and *Hybognathus*)

| Rank | GMAV | ln(GMAV) | ln(GMAV) ² | P=R/(n+1) | √P |
|------|----------|-----------|-----------------------|-----------|-----------|
| 1 | 60.34101 | 4.100012 | 16.8101 | 0.0217391 | 0.147442 |
| 2 | 62.15 | 4.129551 | 17.05319 | 0.0434783 | 0.2085144 |
| 3 | 65.295 | 4.178916 | 17.46334 | 0.0652174 | 0.255377 |
| 4 | 79.99 | 4.381902 | 19.20106 | 0.0869565 | 0.2948839 |
| Sum | | 16.790381 | 70.52769 | 0.2173913 | 0.9062173 |

$$S^2 = [70.52769 - (16.790381)^2/4] / [0.02173913 - (0.9062173)^2/4] = 4.0108$$

$$S = 2.0027$$

$$L = [16.790381 - (2.0027)(0.9062173)]/4 = 3.7439$$

$$A = (2.0027)(\sqrt{0.05}) + 3.7439 = 4.1917$$

$$\text{FAV} = e^{4.1917} = 66.13$$

Table 3

+1 S.E. (+10% mean for GMAVs with only one test value, i.e. *Fluminicola* and *Hybognathus*)

| Rank | GMAV | ln(GMAV) | ln(GMAV) ² | P=R/(n+1) | √P |
|------|---------|-----------|-----------------------|-----------|-----------|
| 1 | 68.35 | 4.224641 | 17.8476 | 0.0217391 | 0.147442 |
| 2 | 79.0795 | 4.370454 | 19.10086 | 0.0434783 | 0.2085144 |
| 3 | 82.779 | 4.416174 | 19.5026 | 0.0652174 | 0.255377 |
| 4 | 148.01 | 4.99728 | 24.97281 | 0.0869565 | 0.2948839 |
| Sum | | 18.008549 | 81.42387 | 0.2173913 | 0.9062173 |

$$S^2 = 28.7086$$

$$S = 5.3580$$

$$L = 3.2882$$

$$A = 4.4863$$

$$FAV = e^{4.4863} = 88.79$$

Chronic Values based on Error Estimates

The Final Chronic Value (FCV) with error estimates for Mill Creek recalculation was derived in Richards (2016) report and was equal to the FAV divided by the geometric mean (with error estimates). The FCV reported in Richards (2016) was 5.319 (3.248, 8.712, 95% CIs). The Criterion Continuous Concentration (CCC) continued to be equal to the FCV = 5.319 (3.248, 8.712). The Criterion Maximum Concentration (CMC) was equal to one-half the Final Acute Value = $0.5 \times 66.13 = 33.07$ for lower estimate and $0.5 \times 88.79 = 44.40$ for upper estimate. The mean CMC reported by Richards (2016) was from 33.45 to 37.64, depending on which taxa were included.

Discussion

It was obvious that although geometric means are likely the most appropriate point estimate method for toxicity testing because sensitivity values are often on a log scale; using only a single value (geometric mean) could be uninformative and possibly misleading. Relying on values based on only one or two tests on a species also led to large error estimation or in the case of one test on a species, no error estimation could be made. This is disconcerting because geometric means used in criteria development based on one or two tests on a species could be far from precise. In addition, the error estimates in this addendum were very conservative. Typically, in ecological studies, error estimates include two standard deviations and 95% CIs (see Figures 1 and 2).

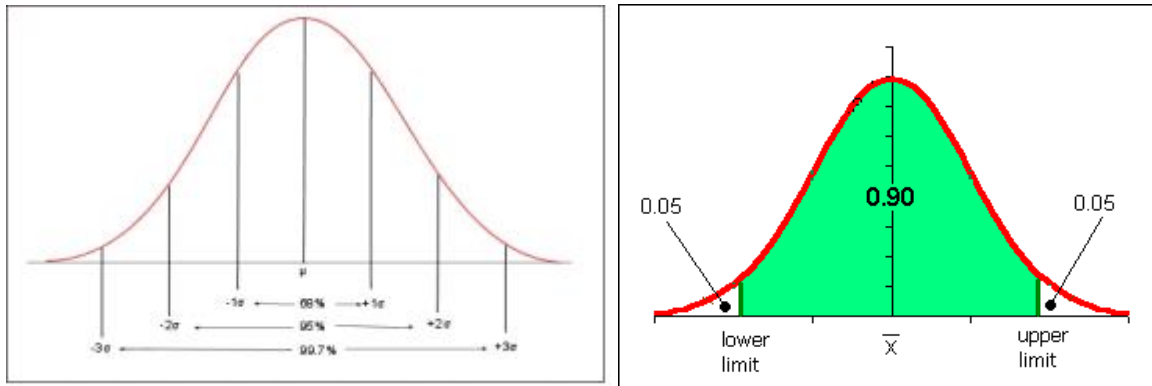


Figure 1. Example of standard errors (left graph) and 90% confidence intervals (right graph) around the mean of a normal distribution. 1 SE = 68% of the values around the mean estimate, 2 SEs = 95% of the values around the mean. 90% confidence intervals around the mean are in green (right graph), 5% on each side of the mean. SMAVs in this addendum were based on geometric means assuming log sensitivities, therefore the transformed data should approximate the normal distribution shown above.

Conclusion

By calculating and reporting error estimates, a better understanding of the values that should be used in ammonia criteria development for Mill Creek and Central Valley Water Reclamation Facility can be employed. Error estimates were not made for other Genus Mean Acute Values (GMAVs) in the recalculation procedure for Mill Creek (Richards 2016) but the error estimates reported in this addendum suggest further calculations may be necessary.

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