| Comment Number | Test Guideline Paragraph (¶) | Comments |
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| 1 | | This threshold approach seems reasonable, although some adjustment of the conclusion when no mortality occurs at step 2 will likely be necessary. As discussed below, there are concerns with the probability calculations, and the confidence intervals generated for the underlying probability, that may warrant incorporation of an allowance or factor above the actual threshold concentration (TC) when the decision not to carry out further fish testing is made.<br><br>The following comments interpret the Threshold Approach as follows: a daphnia or similar first step test (this is where Test Guideline [TG] 201 and TG 202 are relevant) is used preliminarily to set a TC. If that concentration is greater than 100 mg/L, the limit test described in TG 203 (see par. 20 of TG 203), where 7 fish are exposed at 100 mg/L and 7 fish run as controls, is performed.<br><br>Note that by par. 20 of TG 203, if any deaths occur in the limit test, the full 5-or-more-dose-plus-control test is run with at least 7 per group. Par. 6 of the proposal does NOT say to run a full test, only a limit test, at the threshold concentration, despite the possibility that a test concentration appreciably under 100 mg/L may be chosen based on par. 5 of the Threshold Approach proposal. This quite likely will cause problems with classification, owing to the interpretation of the conclusion from TG 203, par. 20, and the relation of the chosen test concentration to the classification cut points.<br><br>Note also, however, the current TG 203 wording permits a spacing as great as the 3.2 that Hoeger et al. (2006) suggested in the (not-proposed) Step-down Approach. This is because par. 17 of TG 203 says "with a factor preferably not exceeding 2.2" but does not give an upper limit on the factor, which could, in fact, be greater than 3.2 if feasible), provided the test remains valid (par. 6), mortalities are not excessive (par. 21 & 22 of TG 203) and 5 groups are maintained (par. 17 of TG 203). The difference between the two Approaches seems to be the use of the inferred threshold from the invertebrate to bound the testing.<br><br>Here are some considerations.<br><br>1) Suppose the TC is something well under 100 mg/L. In particular, suppose it's 5 mg/L. Then all the limit test outcome permits one to say is that there's a high probability that the fish $LC_{50}$ > 5 mg/L. Five mg/L is sufficiently far from 100 mg/L in the scale that's usually |

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| | | tested that one would be hard pressed to say the test gives evidence there's a more sensitive species than the fish, which would be the desired type of evidence according to par. 1.<br><br>2) When a single group test is run with 0/7 mortalities, the exact 95% confidence interval for the underlying probability of mortality ranges from 0 to 41%; the upper limit is not appreciably different from 50%. With 8 in each group, the upper limit is 37%, with 9, 34%, and with 10, 31%. When two groups (one tested at TC, the other control) both show 0/x mortalities, there's a 50-50 chance one proportion is greater than the other, regardless of the exposure assurance that the dosed group is tested at a concentration greater than the control. That suggests to me that the step 2/no-mortality conclusion (per Table of par. 4) is problematic.<br><br>What is needed is the probability that the interval bounded below by (but not including) the lowest dose tested (assuming that's the lowest response, too) includes the LC50. Just because the probability of seeing 0/n at the median dose, for n=7, 8, or 9 is <1% (0.8%, 0.4%, and 0.2%, respectively), as described in par. 20 of TG 203 (when testing if 100 mg/l is the median), doesn't mean that it's the probability that that tested dose is not greater than the median dose. Consequently, the probability statement of par. 6 of the Threshold Approach appears to be a misstatement, and par. 20 of TG 203 may need reexamination to see if its basis is properly stated (its calculation method is unclear).<br><br>This discussion appeals to theory about confidence intervals for quantiles (here, a confidence interval for the value with median response). Point 2) above gives a confidence interval for the underlying probability of failure (p), given that no failures (deaths) were seen with specified n. If one is at the median value, then you would expect p=0.5. But it's the doses that are to be bracketed, not the responses. So it may be better to look at the interval for the quantile (here, 50%ile) to ensure a correct conclusion is made. |
| 2 | 3 | Replace thereafter with hereafter; replace Euroepan with European. |
| 3 | 4 | Replace Figure with Table-- "The following step-wise procedure should be utilized (Figure): |