1. The submitted test method should relate to regulations or guidelines in Japan.

With regard to toxic substances, a notification* in reference to the Poisonous and Deleterious Substances Control Act recommends that OECD Guideline for the Testing of Chemicals, Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method, or Test No. 425: Acute Oral Toxicity - Up and Down Procedure be used in place of Test No. 401 to determine classifications as a poisonous or deleterious substance. This test method is related to that issue.

*NB1: *Notification No. 1209001 of the Office of Chemical Safety, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated December 9, 2002 and titled "Tests necessary to determine classifications as a poisonous or deleterious substance under the Poisonous and Deleterious Substances Control Act."*

With regard to new chemical substances, a notification* in reference to the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture specifies that, when acute toxicity tests are used in preliminary testing for repeated dose toxicity studies, it is desirable to refer to OECD Test Guidelines for test methods. This test method is related to that issue.

*NB2: *Notification No. 1121002 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated November 21, 2003 as well as Notification No. 2 of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry and Notification No. 031121002 of the Environmental Health Department, Environmental Policy Bureau, Ministry of the Environment, dated November 13, 2003, and titled "Test Methods for New Chemical Substances." (Final revision dated November 20, 2006)*

With regard to agricultural chemicals, a notification* in reference to single-dose toxicity tests and table of guidelines for creation of test records for submittal with application for registration of agricultural chemicals, recommends both the Fixed Dose Procedure and the Acute Toxic Class Method. This test method is related to that issue.

*NB3: *Notification No. 12-8147 of the Agricultural Production Bureau of the Ministry of Agriculture, Forestry and Fisheries, dated November 24, 2000 and titled "Test results related to application for registration of agricultural chemicals."*

With regard to drugs, enforcement regulations appurtenant to the Pharmaceutical Affairs Act specify required documentation for submittal with applications for approval of manufacturing or importing drugs. Single-dose toxicity tests are included in the required documentation, the data for which can be obtained using methods that are ordinarily called "acute toxicity tests." A notification* in reference to this specifies techniques for performing single-dose toxicity tests. In the notification, an overview of lethal doses is called for but there is no requirement for a median lethal dose of LD50.

*NB4: *Notification No. 88 of the New Drugs Division, Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, dated August 10, 1993 and titled "Revisions to guidelines for single-dose and repeated-dose toxicity studies."*

With regard to medical devices, an absence of extracts that indicate acute toxicity is called for. This requirement, however, calls only for extraction liquid prepared with either physiological saline or vegetable oil as an extraction medium and does not require that the median lethal dose of LD50, which means that it is unrelated to this test method.

NB5: *Notification No. 0213001 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Heath, Labour and Welfare, dated February 13, 2003 and titled "Basic concepts for biological safety tests necessary to application for approval of manufacturing or importing medical devices" as well as Notification No. 36 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Heath, Labour and Welfare, dated March 19, 2003, and titled "Reference documentation regarding basic concepts for assessment of biological safety."*

With regard to rubber closures for aqueous infusions, section 7.03 of the 15th edition of the Japanese Pharmacopeia (JP 15) calls for acute toxicity testing. That, however, is a constant dose test and is unrelated to this test method.

2. The submitted test method and supporting validation data should have been subjected to a transparent and independent peer review process.

This test method has not been experimentally validated in Japan. Overseas, however, both NICEATM and ECVAM have validated this test method for 72

chemical substances during the period from 2002 to 2005. These validation activities were published in a Background Review Document (BRD).[3]

3. The data generated by the test method should adequately measure or predict the endpoint of interest. For replacement test methods, the data should show a linkage between the proposed test method and an existing test method, and/or the proposed test method and effects in the target or model species.

   This test method is an alternative method for establishing initial doses rather than an alternative to the entire conventional test.

4. The test method should generate data useful for hazard/risk assessment purposes.

   For the same reason as mentioned in item 3, this test method is indirectly useful but is not a direct means of hazard assessment.

5. The submitted test method and supporting validation data should adequately cover a spectrum of chemicals and products representative of those administered by the regulatory program or agency for which the test method is proposed, and the applicability and limitations of the test method should be clearly described.

   This test method is not intended to assure the safety of chemical substances. It is clearly not suitable for the following:

   - For expressions of toxicity activated by metabolism
   - For expressions of toxicity per neurotoxic, cardiotoxic, or other specific mode of action
   - For substances that are insoluble in cell culture medium, that are volatile, or that exhibit a specific effect on lysomes
   - For substances with coloration that duplicates the color absorbency of neutral red

6. The test method should be sufficiently robust (relatively insensitive to minor changes in protocol) and transferable among properly-equipped laboratories with adequately-trained staff.

There is no experimental data to substantiate any claim of robustness for the protocol used in this test.
The test procedure itself, however, is easily transferrable to any properly-equipped laboratory with an adequately-trained staff.

7. The test method should be both time and cost effective as well as likely to be used in a regulatory context.

Other than simulations, there is no quantitative data that conclusively demonstrates the time and cost effectiveness of this test method. Thus, the effectiveness of this test method has yet to be clarified in quantitative terms.

Because the effectiveness of this test method has yet to be clarified in quantitative terms, it is unlikely to be used in a regulatory context.

8. Justification should be provided (scientific, ethical, economical) for the new or updated test method in light of existing test methods.

The existing test is used to evaluate somatic death, in which there exist several mechanisms. This test method, however, makes evaluations by indexing cell death, which means that there are limits to its scientific justification.

That being said, compared with existing test methods, using this test method to establish initial doses does not result in conclusively adverse effects, which means that there is scientific justification with the those limited parameters.

This is a new proposal ethically, but the economic justification is as of yet unclear.

9. Is this test method acceptable for use in regulatory documentation on safety assessment?

This test method is a means of establishing initial doses for acute toxicity tests.

Based on the above, the JaCVAM Regulatory Acceptance Board has determined the following for this alternative method for single-dose toxicity test methods.

There is no major benefit to justify the use of this test method.

There are some advantages to the use of this test method, and its disadvantages are not fatal.

A regulatory proposal that recommends without compelling the use of this test method would conform to the principles of 3R.