

RATIONALE FOR THE UP-AND-DOWN PROCEDURE AS SUBMITTED TO THE OECD

Introduction

1. Acute toxicity tests are used to evaluate various toxic manifestations following a single exposure to an agent. One of the uses of data coming from such tests is to estimate the median lethal dose so as to place agents into one of a number of groups for hazard classification and labeling purposes. OECD presently has approved three test methods for acute oral toxicity: Test Guideline 401: the classical Acute Toxicity Test, and two substitutes, Test Guideline 420 the Fixed Dose Method (FDM) and Test Guideline 423: the Acute Toxic Class Method (ATC). The Up-and-Down Procedure (UDP) would be a fourth such option.

Background

2. All of the acute oral toxicity tests measure a spectrum of non-lethal toxic manifestations. Both the classical method (TG 401) and the UDP give point estimates of the median lethal dose, whereas the FDM (TG 420) and ATC (TG 423) give estimates of the lethal range. The classical test relies on simultaneous testing of a preset number of groups of animals, while the other three tests employ consecutive testing in a staircase design, where the dose in one trial is a function of the outcome of testing in the previous trial. The UDP and the ATC are quite consistent, except that the UDP uses single animals per trial, while the ATC employs three animals per dose.

3. Significant work has been performed on the UDP. Theoretical studies have demonstrated the characteristics of the method and indicated that the procedure and its modifications are very efficient means of deriving an estimate of the median effective dose per expenditure of test animals (1)(2)(3)(4)(5)(6). Practical determinations of acute toxicity bear this out, where savings in animals in comparison to the classical test and the FDM can be significant; the UDP and the ATC appear to use quite comparable numbers of animals (1)(7)(8)(9)(10)(11)(12). In addition, practical use of the test method goes far beyond acute toxicity testing and includes such things as (a) evaluation of target organ effects in dogs (13); (b) evaluation of the efficacy of antiemetic drug treatments (14); determination and treatment of adverse organophosphate-induced effects (15)(16)(17); and (d) testing of the movement of chemicals imbedded in microspheres through the human stomach (18).

4. Before being accepted by OECD the FDM and the ATC each underwent validation ring tests. Validation of a new method depends upon determining the reliability and reproducibility of the method, proving its predictive capacity, and establishing its relevance. Since data on the UDP demonstrate all of these, it seems to be both unnecessary and undesirable to undertake extensive validation testing of this method.

Reliability and Reproducibility

5. The test method for the UDP is like that used in the classical test, FDM and ATC: the species of animal used is the same; the method of administration of the test material is the same; and the observations and toxic endpoints are the same. These ensure that the animal data gathered by a laboratory for the UDP are just like those from the other acute toxicity test methods that have

already been adopted as OECD Test Guideline. Further validation of the UDP to demonstrate that multiple laboratories can reliably administer test substances to experimental animals and determine acute toxicity manifestations including whether they survive or die is not necessary.

Predictivity

6. Acute toxicity findings using the UDP have been generally similar to those achieved with the classical method: there was an excellent linear correlation for the estimates of the median lethal dose, and the same EEC acute toxicity classification was reached in 23 of 25 cases (12). In the two remaining cases, the UDP classification was more stringent than the classical method. These data on 25 test materials clearly indicate that the UDP can predict the appropriate hazard classes of test materials as well as the classical method. In addition, the mathematical model used in the UDP to predict the median lethal dose of test materials has been published as an American Society for Testing and Materials standard method (19).

7. Both the FDM and the ATC were found acceptable after testing 20 chemicals, a number similar to that accumulated in multiple studies for the UDP (11)(12)(20). In addition, FDM, ATC and UDP testing led to the same hazard classification decisions as did the classical test in 80, 85 and 92% of cases, respectively. Certainly, the data base supporting the UDP is comparable to other methods that have been accepted by OECD Member countries.

Relevance

8. Test methods must be relevant to the regulatory agencies that are going to use the test data. As stated previously, the UDP has become a standard test method by the American Society for Testing and Materials (ASTM, 1987). In addition to capturing all of the toxic manifestations following acute exposure to an agent, the UDP test provides an estimate of the median lethal dose which is directly referable to any hazard classification system in use today. Such an estimate of the median lethal dose is also often helpful in setting doses for subchronic toxicity tests and for comparisons of acute toxicity with other test materials and by other routes of administration.

9. Regulatory agencies are also concerned about the use of animals in toxicity tests. The UDP has been shown to use fewer animals than the classical test and the FDM, and while a direct comparison between the UDP and ATC method is only available for three materials, the UDP used either the same or fewer animals (Schlede et al., 1994; Lipnick, et al., 1995). The UDP provides in a single test the ability to correctly classify acute toxicity as well as to estimate the median lethal dose, data that can be useful in preventing unnecessary animal use in future toxicity studies.

Conclusion

10. All acute toxicity tests are trying to develop the same data on the consequences of a single chemical exposure: they measure morbid endpoints and lethality. Like other acute toxicity tests, the UDP can be used to reliably and reproducibly evaluate acute toxicity. Methods differ in regard to details of their design and means of determining values used for hazard classification. Certainly the UDP is as efficient a means of estimating a median lethal dose as exists. It predicts an appropriate hazard classification as well as other acute toxicity alternatives, and its relevance to

regulatory objectives is ably demonstrated by developing requisite toxicity data, estimating the median lethal dose and minimizing animal usage. To commit more animals in order to show that the method works would be contrary to good science, good policy and good economics.

References

1. Brownlee, K.A., Hodges, J.L. & Rosenblatt, M. 1953 The up-and-down method with small samples. *J. Amer. Statist. Assn.* 458: 262-277.
2. Wetherill, G.B., Chen, H. & Vasudeva, R.B. 1966 Sequential estimation of quantal response curves: A new method of estimation. *Biometrika.* 53: 439-454.
3. Dixon, W.J. 1965 The up-and-down method for small samples. *J. Amer. Statist. Assoc.* 60: 967-978.
4. Hsi, B.P. 1969 The multiple sample up-and-down method in bioassay. *J. Amer. Statist. Assoc.* 64: 147-162.
5. Little, R.E. 1974a A mean square error comparison of certain median response estimates for the up- and-down method with small samples. *J. Amer. Statist. Assoc.* 69: 202-206.
6. Little, R.E. 1974b The up-and-down method for small samples with extreme value response distributions. *J. Amer. Statist. Assoc.* 69: 803-806.
7. Bonnyns, E., Delcour, M.P. & Vral, A. 1988 Up-and-down method as an alternative to the EC-method for acute toxicity testing. Brussels: Institute of Hygiene and Epidemiology, Ministry of Public Health and the Environment. IHE project no. 2153/88/11. 33 pp.
8. Bruce, R.D. 1985 An up-and-down procedure for acute toxicity testing. *Fundam. Appl. Toxicol.* 5: 151-157.
9. Bruce, R.D. 1987 A confirmatory study for the up-and-down method for acute toxicity testing. *Fundam. App. Toxicol.* 8: 97-100.
10. Yam, J., Reer, P.J. & Bruce, R.D. 1991 Comparison of the up-and-down method and the fixed dose procedure for acute oral toxicity testing. *Fd. Chem. Toxicol.* 29:259-263.
11. Schlede, E., Diener, W., Mischke, U. & Kayser, D. 1994 OECD expert meeting: Acute toxic class method. January 26-28, 1994, Berlin, Germany.
12. Lipnick, R.L., Cotruvo, J.A., Hill, R.N., Bruce, R.D., Stitzel, K.A., Walker, A.P., Chu, I., Goddard, M., Segal, L., Springer, J.A. & Myers, R.C. 1995 Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. *Fd. Chem. Toxicol.* 33: 223-231.

13. Klaasen, C.D. & Plaa, G.L. 1967 Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. *Toxicol. Appl. Pharmacol.* 10: 119-131.
14. Cordts, R.E. & Yochmowitz, M.G. 1983 Antiemetic studies both pre and post exposure: Preliminary findings. Report USAFSAM-TR-83-23. Brooks Air Force Base, TX: USAF School of Aerospace Medicine. 9 pp.
15. Blick, D.W., Murphy, M.R., Weathersby, F.R., Brown, G.C., Yochmowitz, M.G., Fanton, J.W., & Harris, R.K. 1987a Primate equilibrium performance following soman exposure: Effects of repeated daily exposure to low soman doses. Report USAFSAM-TR-87-19. Brooks Air Force Base, TX: USAF School of Aerospace Medicine. 18 pp.
16. Blick, D.W., Murphy, M.R., Brown, G.C., Yochmowitz, M.G., & Farrer, D.N. 1987b Effects of carbamate pretreatment and oxime therapy on soman-induced performance decrements and blood cholinesterase activity in primates. Report USAFSAM-TR-87-23. Brooks Air Force Base, TX: USAF School of Aerospace Medicine. 12 pp.
17. Blick, D.W., Murphy, M.R., Brown, G.C. & Yochmowitz, M.G. 1987c Primate equilibrium performance following soman exposure: Effects of repeated acute exposure with atropine therapy. Report USAFSAM-TR-87-43. Brooks Air Force Base, TX: USAF School of Aerospace Medicine. 11 pp.
18. Meyer, J.H., Elashoff, J., Porter-Fink, V., Dressman, J. & Amidon, G.L. 1988 Human postprandial gastric emptying of 1-3 millimeter spheres. *Gastroenterology.* 94: 1315-1325.
19. ASTM 1987 (American Society for Testing and Materials) Standard test method for estimating acute oral toxicity in rats. Designation: E 1163-87. Philadelphia: American Society for Testing and Materials.
20. Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Oliver, G.J.A., Pelling, D., Tomlinson, N.J. & Walker, A.P. 1990 The international validation of a fixed-dose procedure as an alternative to the classical LD50 test. *Fd. Chem. Toxicol.* 28: 469-482.