

# Acute Oral Toxicity

K.S. Rao\*

## INTRODUCTION

I am covering this simple and mundane acute toxicity test to increase the awareness, understanding and an appreciation of acute toxicity test, which has undergone sea-change in the last decade. In this commentary, I am not going to discuss details of different OECD guidelines which any one can download from the OECD website.

The conventional acute oral toxicity test (Formerly OECD Test Guideline 401) involving treatment of multiple simultaneous dose groups of animals of both sexes which used over 40 rats, which was the most heavily criticized test in terms of animal welfare. This concern was the driving force behind the development of three alternative tests for acute oral toxicity (Test Guidelines 420, 423, 425). Anticipating the presence of validated alternatives, OECD Member countries took the initiative of deletion of OECD Guideline 401.

Acute oral toxicity data are used to satisfy hazard classification and labeling requirements, for risk assessment for human health and the environment and when estimating the toxicity of mixtures. The provision of either a point estimate of the LD<sub>50</sub> value or range estimate of the LD<sub>50</sub> generally meets the acute oral toxicity data requirements for classification for all regulatory authorities in the areas of industrial chemicals, consumer products and for many pesticide applications.

For reasons of animal welfare concern, testing of animals in Globally Harmonized System (GHS) category 5 ranges (2000-5000 mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

Acute oral toxicity testing by OECD methods is not required for pharmaceuticals.

Pharmaceutical methods are specified by the International Committee on Harmonization (ICH). In some specific cases such as imaging and antineoplastic agents, estimates of acute toxicity are needed to support single dose studies in human. These studies call for testing to fully characterize the toxicity in the low toxicity region and may involve doses above 2000 mg/kg. However, the study designs for these

special purpose studies are different from any of the current OECD acute toxicity study guidelines.

## Comparison of OECD Guidelines 420, 423 and 425

### i. Outline of the Methodology

All of the guidelines involve the administration of a single dose of test substance to fasted healthy young adult rodents by oral gavage, observation for up to 14 days after dosing, recording of body weight and the necropsy of all animals.

### ii. Dosing/Treatment

Doses may be administered based on a constant volume or a constant concentration depending upon the needs of the toxicologist and the regulatory authorities. Some authorities prefer that substances sold to the public should be tested as constant concentration unless the volumes are too small to administer accurately. Since the effects at the same dose may be different if the materials are diluted, it is important for the toxicologist to consider how the information will be used. If the material will primarily be used, diluted in mixtures, then constant volume may be appropriate.

On the other hand, if the material is to be used neat, particularly if it may be irritating, the use of constant concentration will be more appropriate. Each animal should be selected from the available animals in a random fashion on the day of dosing. In recognition of the fact that most animal suppliers do not indicate littermates, the guidelines do not call for randomizing animals from a single litter across dose groups.

### iii. Study Details

At the commencement of its dosing, each animal should be between 8 to 12 weeks age and its body weights should fall in an interval within  $\pm 20\%$  of the mean body weight of all previously dosed animals taken on their day of dosing. As the mean weight will increase as the animals age, this method tends to correct for the change in animals weights with time. In order to conform to this age and weight requirements at the start of dosing of each animal, it may be necessary to order animals sequentially as the tests can sometimes take several weeks to complete. The

---

## K.S. Rao

Advinus Therapeutics Ltd, 21 and 22,  
Phase II, Peenya Industrial Area, Bengaluru – 560058, Karnataka, INDIA.

### Correspondence

**Dr. K.S. Rao, (M.V.Sc., Ph.D., DABT)**

Senior Director – Strategic Business Development, Advinus Therapeutics Ltd, 21 and 22, Phase II, Peenya Industrial Area, Bengaluru – 560 058, Karnataka, INDIA.

Ph.no: +91-733-783-0074

Email: ks.rao@advinus.com

### History

- Submission Date: 10-11-2018;
- Review completed: 14-12-2018;
- Accepted Date: 16-12-2018.

**DOI : 10.5530/bems.4.2.10**

### Article Available online

<http://www.bemsreports.org>

### Copyright

© 2018 Phcog.Net. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

**Cite this article :** Rao KS. Acute Oral Toxicity. BEMS Reports. 2018;4(2):39-41.

primary endpoint for Guidelines 423 and 425 is mortality, but for Guideline 420 it is the observation of clear signs of toxicity (termed: evident toxicity).

#### iv. OECD Guideline 420

A sighting study is included for Guideline 420 in order to choose an appropriate starting dose and to minimize the number of animals used. Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used both in the sighting study and the main study. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity.

Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of signs of toxicity, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing evident toxicity, except when there are no effects at the highest fixed dose.

#### v. OECD Guideline 423

Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce mortality in some animals.

Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of mortality, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing mortality, except when there are no effects at the highest fixed dose.

#### vi. OECD Guideline 425

This is also a stepwise procedure, but uses single animals, with the first animal receiving a dose just below the best estimate of the LD<sub>50</sub>. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by a factor of 3.2. This sequence continues until there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival, or decreasing dose results in survival rather than death); then, additional animals are dosed following the Up-Down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then no more than a specified number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/kg should be taken only when justified by a specific regulatory need.

### Animal Welfare Considerations

All three Guidelines provide significant improvements in the number of animals used in comparison to Guideline 401, which required 20 animals in a test, at least. In addition, they all contain a requirement to follow the OECD Guidance Document on Humane Endpoints, which should reduce the overall suffering of animals used in this type of toxicity test.

Furthermore, Guideline 420 has as its endpoint evident toxicity rather than mortality and uses a sighting study to minimize the numbers of animals and Guideline 425 has a stopping rule, which limits the number of animals in a test.

#### i. OECD Guideline 420

Groups of five young adult animals of one sex are dosed per step in the main study. Single animals are used per step in the sighting study. Regulatory experience and statistical modeling has shown that most tests are likely to be completed with either one or two sighting study steps or one

main study step, thus using between 5 and 7 animals. Up to 5 animals are used in a limit test.

#### ii. OECD Guideline 423

This test uses groups of 3 animals of one sex per step. Regulatory use of this Guideline demonstrates that the average number of animals used is 7. Up to 6 animals are used in a limit test.

#### iii. OECD Guideline 425

This test uses single animals of one sex. Statistical modeling indicates that the average number of animals used in this test is about 6-9. Up to 5 animals are used in a limit test. The following estimates of the number of treatment related deaths for tests conducted on substances with LD<sub>50</sub> values below 5000 mg/kg are based on practical experience and validation studies using earlier versions of these guidelines and statistical modeling.

#### iv. OECD Guideline 420

Typically, 1 animal can be expected to die on test.

#### v. OECD Guideline 423

Two to three animals per test can be expected to die in a full test.

#### vi. OECD Guideline 425

The expected number of deaths is between 2 and 3.

#### vii. Observations

For all three guidelines, careful clinical observations should be made at least twice on the day of dosing or more frequently when indicated by the response of the animals to the treatment and at least once daily thereafter. Additional observations are made if the animals continue to display signs of toxicity. Observations include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behaviour pattern. Animals that are moribund or suffering severe pain and distress must be humanely sacrificed. Guidance on clinical signs and objective measurements that are indicative of impending death and/or severe pain and/or distress is available in an OECD Guidance Document. Humanely killed animals are considered in the same way as animals that died on test.

### Information Provided by Each Method

OECD Test Guidelines 420 and 423 provide a range estimate of the LD<sub>50</sub>; the ranges are defined by cut-off values of the applied classification system and not as a calculated lower and upper level. In the case of test Guideline 420, this range is inferred from the fixed dose which produces evident toxicity. Guideline 425 provides a point-estimate of the LD<sub>50</sub> value with confidence intervals.

The results of tests conducted according to Guideline 425 will allow a test substance to be classified according to all the systems in current use, including the new GHS. Test Guidelines 420 and 423 have now been revised to allow classification according to the new GHS.

### Limitations of the Methods

Validations against actual data and statistical simulations identified areas where all three methods may have outcomes which result in a more or less stringent classification than that based on the "true" LD<sub>50</sub> value (As obtained by the deleted guideline 401). Comparative statistical analysis indicates that all are likely to perform poorly for chemicals with shallow dose-response slopes.

For all methods, the study outcome is likely to be influenced by the choice of starting dose level(s), relative to the "true" LD<sub>50</sub> value, especially in the case of shallow slopes. Because Guideline 420 uses evident toxicity as an

endpoint instead of death, information on toxic effects seen only at dose levels close to a lethal dose will not always be obtained.

Unusually, test substances may cause delayed deaths (5 days or more after test substance administration). Substances which cause delayed deaths have an impact on the practicality of conducting a study to Guideline 425 where the duration of testing will be significantly longer compared with other test methods. However, both in Guideline 420 and 423, the finding of a delayed death may require additional lower dose levels to be used or a study to be repeated.

### Information Provided by Each Method

Test Guidelines 420 and 423 provide a range estimate of the LD<sub>50</sub>; the ranges are defined by cut-off values of the applied classification system and not as a calculated lower and upper level. In the case of Test Guideline 420 this range is inferred from the fixed dose which produces evident toxicity. Guideline 425 provides a point-estimate of the LD<sub>50</sub> value with confidence intervals.

The results of tests conducted according to Guideline 425 will allow a test substance to be classified according to all the systems in current use, including the new GHS. Test Guidelines 420 and 423 have now been revised to allow classification according to the new GHS. However, in order to cover the transition period until the global implementation of the GHS both Guidelines also allow classification according to existing systems.

### Optimizing the Performance of the Test

a. Each guideline provides procedures to assist in selecting the starting dose, particularly in the event that minimal prior information on the substance itself is available. All available information on the test substance must be made available to the testing laboratory and should be considered prior to conducting the study. Such information will include, for example, the identity and chemical structure of the substance; its physico-chemical properties; the result of any other *in vivo* or *in vitro* toxicity tests on the substance; toxicological data on structurally related substances; the anticipated use(s) of the substance; and the likely regulatory data requirements.

This information is necessary to satisfy all concerned that the test is relevant for the protection of human and animal health and mammalian wildlife, to select the most appropriate test to satisfy regulatory requirements and will help in the selection of the starting dose.

b. For all three methods the efficiency of the test, in terms of reliability and numbers of animals used, is optimized by the choice of a starting dose close to (423) or just below (425) the actual LD<sub>50</sub> or the lowest dose producing evident toxicity (420). When this type of information is not available, all three Guidelines include advice on the starting dose level which should be used to minimize the possibility of biased outcome and adverse effects on animal welfare. As a

general principle it is suggested that a starting dose is selected that is slightly lower than the best estimate of the LD<sub>50</sub> based on available evidence.

c. The Limit Test is an efficient way to characterize substances of low toxicity when there is sufficient information available indicating that the toxic dose is higher than the limit dose. Each method provides a limit test suitable to the design of the main study. A Limit Test should be conducted only when there are strong indications that the test substance is of low or negligible acute toxicity.

### Use of Single Sex

a. Guidelines 420, 423 and 425 are conducted using a single sex in order to reduce variability and as a means of minimizing the number of animals used. Normally females are used. This is because literature surveys of conventional LD<sub>50</sub> tests show that usually there is little difference in sensitivity between the sexes but, in those cases where differences were observed, females were generally slightly more sensitive.

b. For chemicals which are direct acting in their toxic mechanism, this may be because female rats have a lower detoxification capacity than males, as measured by specific activity of phase I and II enzymes. However, all available information should be evaluated, for example on chemical analogues and the results of testing for other toxicological endpoints on the chemical itself, as this may indicate that males may be more sensitive than females. Knowledge that metabolic activation is required for a chemical's toxicity can also indicate that males may be the more sensitive sex.

Occasionally, the results of subsequent testing, for example a sub-chronic test, may raise concerns that the more sensitive sex had not been used. In such cases and only when considerable differences between the sexes are suspected, it may be necessary to conduct another full acute oral toxicity study in the second sex. This is preferable to conducting confirmatory testing in a small group of animals of the second sex as a late satellite to the original test because there is a strong possibility that this would produce results that are difficult to interpret.

c. The impact of conducting a second full test on the overall number of animals used in acute toxicity testing should be small because re-testing is anticipated to be infrequent and the results of the test in one sex, together with data from any subsequent studies, will greatly assist in the selection of starting doses closer to the LD<sub>50</sub> in the second test.

### Disclaimer

This newsletter is solely intended for educational purpose. Some of the contents of this newsletter may have been adopted without or with modification from other published resources.

**Cite this article :** Rao KS. Acute Oral Toxicity. BEMS Reports. 2018;4(2):39-41.