Review Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

OECD GUIDELINES FOR ACUTE ORAL TOXICITY STUDIES: AN OVERVIEW

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Received on: 29/06/23 Accepted on: 20/07/23

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DOI: 10.7897/2277-4343.1404130

ABSTRACT

The OECD Guidelines for testing chemicals provide a unique method for assessing the potential impact of chemicals on human health and the environment. These are divided into five sections. Oral toxicity studies are mentioned in the fourth section, i.e. Health Effects. This section cites the guidelines for acute, sub-acute, sub-chronic and chronic oral toxicity studies. Acute oral toxicity refers to those adverse effects of oral administration of a single dose or multiple doses given within 24 hours. OECD guidelines for Acute oral toxicity include 420 (Fixed Dose Procedure), 423 (Acute Toxic Class Method) and 425 (Up-and-Down procedure). OECD guidelines 420, 423 and 425 provide information on the hazardous properties and allow the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity. In OECD Guideline 423, the method classifies the test substance as one of a series of toxicity classes defined by fixed LD_{50} cut-off values, while the OECD Guideline 425 method permits estimation of LD_{50} with a confidence interval. Based on these guidelines, a toxic dose of the drug is obtained. This study aims to compare and highlight the acute oral toxicity guidelines.

Keywords: OECD Guideline, Toxicity study, Acute oral toxicity, LD_{50}, GHS category

INTRODUCTION

The OECD guidelines for the testing of chemicals are internationally accepted specifications for the testing of chemicals decided by the Organisation for Economic Co-operation and Development (OECD) for safety testing. The guidelines are used by experts in industry, academia and government involved in the testing and assessment for hazards of chemicals (industrial chemicals, pesticides, personal care products, etc.) [1]. A total of 185 guidelines are mentioned in the list of test guidelines published in July 2022 and are divided into five sections for their study. Section 1 describes Physical and Chemical properties of the chemicals which includes 25 guidelines (101- 125). Section 2 narrates the Effects of chemicals on the Biotic System, comprising 51 guidelines (201-251). Section 3 illustrates the Environmental fate and Behaviour of chemicals, which covers 20 guidelines (301-320). Section 4 comprises Health effects of chemicals which contains 80 guidelines (401- 498). In section 4, nine guidelines are omitted, viz. 434, 454, 459, 461, 462, 463, 464, 465, and 466. Section 5 narrates Part A: Pesticide Residue Chemistry, which includes 9 guidelines (501-509) [2].

Toxicity studies are conducted for safety assessment and to determine the possible adverse effects of a test substance [3]. For establishment of new drug it is important to perform toxicity studies which is part of drug development phase. Acute, sub-acute and chronic toxicity studies are included in non-clinical safety study [4]. So, the formulation's toxic characteristics, safety, and toxic doses will be known. The test result allows a substance to be ranked and classified according to the Globally Harmonised System for classifying chemical substances and mixtures. The OECD test guidelines are recognised worldwide as the standard reference tool for chemical testing [5]. In Section-4 OECD guidelines of acute (401, 402, 403, 404, 405, 420, 423, 425, 433, 436), sub-acute (407, 410, 412), sub-chronic (408, 409, 411, 413) and chronic toxicity (451, 452, 453) are mentioned. Guidelines for acute oral toxicities are mainly 420, 423 and 425, respectively.

Acute oral toxicity data are used to satisfy hazard classification and labelling requirements, for risk assessment for human health and the environment and when estimating the toxicity mixtures [6]. The drug development phase of any drug molecule follows these guidelines. However, there is a requirement for Ayurvedic classical formulations to go through these phases. This review study highlights OECD guidelines of acute oral toxicity (420, 423 and 425) for safety evaluation and toxicity assessment of chemicals.

Literature Review

This review extracts scientific data by compiling and comparing OECD guidelines 420, 423 and 425 decided by the Organisation for Economic Co-operation and Development (OECD) for acute oral toxicity studies.

Overview of Guidelines for Acute Oral Toxicity Studies [7-9]

Acute oral toxicity: It refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. The updates and details of the OECD Guidelines for Acute Oral Toxicity Study are summarised in Tables 1 & 2. [10-15]

Principle of the test: The principle of the test for acute oral toxicity used under OECD guidelines is shown in Table 3.

Table 1: Updates on Acute Oral Toxicity Test Guidelines

Toxicity Type	No.	Title	Original Adoption	No. of Updates	Updates	Most Recently Updated
Acute oral toxicity study	401	Acute Oral Toxicity	12 May 1981	1	24 February 1987	Date of Deletion: 17 December 2001
	420	Acute Oral Toxicity: Fixed Dose Procedure	17 July 1992	1	-	17 December 2001
	423	Acute Oral Toxicity: Acute Toxic Class Method	22 March 1996	1	-	17 December 2001
	425	Acute Oral Toxicity: Up-and-Down Procedure	21 September 1998	2	17 December 2001	23 March 2006 (corrected in 2008 & 2022)

Table 2: Summarisation of OECD Guidelines for Acute Oral Toxicity Studies

OECD Guideline No.	Name	Duration	Animal Type	Sex	Animal Used
420	Fixed dose procedure	14 Days	Rodent	Single-sex (normally females)	For each dose level i. Sighting study -1 Animal, ii. Main Study -5 Animals Limit Test - 4 Animals
423	Acute Toxic class method	14 Days	Rodent	Single-sex (normally females)	Main Study -3 Animals For each step Limit Test - i. At a dose of 2000 mg/kg - 6 Animals (3 Animals per step) ii. At dose 5000 mg/kg - 3 Animals
425	Up and Down Procedure	14 Days	Rodent	Single-sex (normally females)	Main Test - Min. 4 to 6 Animals & Max. 15 Animals Limit Test - 5 Animals

Table 3: Principle of the Test for Acute Oral Toxicity Studies

OECD Guideline	Principle of the test
420	Groups of animals are dosed in a stepwise procedure with fixed doses 5, 50, 300 and 2000 mg/kg (exceptionally, an additional fixed dose of 5000 mg/kg is considered) and depending on the presence or absence of signs of toxicity or mortality, animals dosed at higher or lower fixed doses.
423	The substance is tested using a stepwise procedure, each step using three animals. The absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e. no further testing is needed, dosing of three additional animals with the same dose and dosing of three additional animals at the next higher or lower dose level.
425	Single ordered dose progression, animals are dosed one at a time, at a minimum of 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD_{50}. If the animal survives, the dose for the next animal is increased to 3.2 times the original dose. If an animal dies, the dose for the next animal is decreased by a similar factor.

Description of the method: It is common for OECD Guideline 420, 423 and 425 (Table 4).

Table 4: Description of the Method for Acute Oral Toxicity Studies

Selection of animal species	Animal species - Rodent (rat), normally nulliparous and non-pregnant females are used. Age - between 8 - 12 weeks Weight - should not exceed ± 20% of the mean weight of the previously dosed animal. Appropriate justification should be required when testing is performed on males.
Housing and feeding conditions	Temperature - 22ºC (± 3ºC) Humidity - 30% -70% Lighting - Artificial, 12 hours light and 12 hours dark sequence. Feeding - conventional laboratory diets, unlimited supply of drinking water. Animals may be group-caged by dose. At 48 hours after dosing, animals may be returned to group housing unless there are reasons to house them individually (OECD 425).
Preparation of animals	Randomly selected animals with individual identification should be acclimatised in cages for five days prior to dosing.
Preparation of doses	Test substance should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Depending on the size of the test animal, the maximum volume of liquid can be administered at one time. In rodents, volume should not usually exceed 1 mL/100g of body weight (aqueous solutions - 2 mL/100g body weight). With respect to the formulation of the dosing preparations, aqueous solution/ suspension/ emulsion is recommended in order of preference and then possibly solution in other vehicles. The toxicological characteristics of the vehicle should be known (vehicles other than water). Preparation of the doses must be done before administration.

Procedure

Administration of doses for Acute Oral Toxicity Studies (OECD Guideline 420, 423, 425)

- The procedure for administration of doses is seen similar in OECD Guideline 420, 423 and 425.
- The test substance is administered by gavage using an appropriate stomach tube or intubation cannula in a single dose. If a single dose is not possible, the dose can be given in a smaller fraction within 24 hours.

- Animals should be fasted before administration of doses, e.g. food should be withheld overnight and for 3-4 hours in rats and mice, respectively, but water should not be withheld.
- After fasting, the animals should be weighed, and the test substance should be administered.
- The dose is calculated according to the body weight after determining the fasted body weight of each animal.
- After dosing, food may be withheld for 3-4 hours in rats or 1-2 hours in mice. It may be necessary to provide food and water where a dose is administered in fractions over some time, depending on the length of the period.

Table 5: Procedure of Acute Oral Toxicity Studies mentioned in OECD Guideline 420, 423 and 425

	OECD Guideline 420	OECD Guideline 423	OECD Guideline 425
Sighting study	The purpose of the study is to select an appropriate starting dose for the main study.	-	-
Main study	**Numbers of animals and dose levels –** One of three actions required at the starting dose level is either stop testing and assign the appropriate hazard classification class, test at a higher fixed dose or test at a lower fixed dose. At each dose level, 5 animals of single-sex will be used. A period of 3 or 4 days between dosing at each dose level is recommended to observe delayed toxicity.	**Numbers of animals and dose levels –** The starting dose is selected from one of four fixed levels: 5, 50, 300 and 2000 mg/kg body weight. The study is carried out in 2 steps, and three animals are required for each step. The starting dose level should produce mortality in some of the dosed animals. The time interval between treatment groups is determined by the onset, duration and severity of toxic signs.	The dose is selected in the sequence 1.75, 5.5, 17.5, 55, 175, 550, 1750, 2000 or 5000mg/kg for specific regulatory needs. The test stops when one of the following stopping criteria is met first: a) 3 consecutive animals survive at the upper bound, b) 5 reversals occur in any 6 consecutive animals tested, c) At least 4 animals have followed the first reversal, and the specified likelihood ratios exceed the critical value.
Limit Test (for likely to be non-toxic test material)	A sighting study starting dose of 2000 mg/kg or (5000 mg/kg in exceptional cases) followed by dosing of further 4 animals.	At one dose level of 2000 mg/kg body weight - with 6 animals (three animals per step) At one dose level of 5000 mg/kg body weight - with 3 animals. Additional testing at the next lower level may be required if substance-related mortality occurs.	**Limit Test at 2000 mg/kg** – LD_{50} is less than 2000 mg/kg when 3 or more animals die. LD_{50} is greater than 2000 mg/kg if 3 or more animals survive. **Limit Test at 5000 mg/kg** – LD_{50} is less than 5000 mg/kg when 3 or more animals die. LD_{50} is greater than 5000 mg/kg when 3 or more animals survive.

Testing at doses above 2000 mg/kg

Use of an additional upper fixed dose of 5000 mg/kg may be considered only in rare cases and when reasonable by regulatory requirements. For animal welfare reasons, testing animals within the GHS Category 5 (2000-5000 mg/kg) range is not recommended and should only be considered when there is a high probability that the results of such testing are relevant for protecting human or animal health or the environment. Thus, the condition for testing at doses above 2000 mg/kg is given similarly in OECD Guideline 420, 423 and 425.

Observations for Acute Oral Toxicity Studies for OECD Guideline 420, 423 and 425

After dosing, animals should be observed individually during first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total 14 days. Duration of observations depends on toxic reactions, time of onset and length of recovery period and may thus be prolonged when necessary. The times at which signs of toxicity appear and disappear are important because of tendency for delayed toxic signs. Additional observations such as changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, behaviour pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma should be considered. Animals in a moribund condition or showing signs of severe distress should be humanely killed. When animals are humanely killed or found dead, the time of death should be recorded. Observations of individual weights of animals should be taken at 0, 7^{th} and 14^{th} day. Necropsy and histopathological findings of all animals should be recorded. Microscopic examination of organs with gross pathology in animals surviving 24 or more hours should be noted.

DISCUSSION

Toxicity studies are essential for the evaluation of the safety of the formulations. For these studies, OECD guidelines are recommended by the Organisation for Economic Co-operation and Development (OECD). Acute oral toxicity study provides information on toxic doses of test substances. These studies are conducted per the recommendations of OECD Guidelines 420, 423 and 425. The present review article contains a detailed systematic overview of OECD Guidelines 420, 423 and 425 for Acute Oral Toxicity Study.

Fixed dose procedure with fixed doses 5, 50, 300 and 2000 mg/kg, sighting study, use of 5 animals for testing, dosing at higher or lower fixed doses and confirmation of GHS category are the peculiarities of OECD guideline 420.

In OECD guideline 423 directly main test is performed at each dose level 5, 50, 300 and 2000 mg/kg. For every dose level, 2 step is necessary to confirm toxicity class and LD_{50} cut-off values. In the first step, 3 animals are dosed. If 2-3 animals moribund or died, stop testing, which confirms the GHS category and LD_{50} cut-off value. If 0-1 animal moribund or died then 3 animal dosed in second step at same dose level for confirmation. Out of 3 animals, if 2-3 animals moribund or died (at the same dose level), stop testing, which confirms the GHS category and LD_{50} cut-off value. If 0-1 animal moribund or died, go towards the next higher dose and perform the same step. As this guideline confirms toxicity class, it is termed an acute toxic class method.

In OECD guideline 425, different principles are given for the limit and main tests. Limit tests for 2000 mg/kg and 5000 mg/kg are mentioned separately. Dose progression is mentioned in this guideline, which depends on the survival and mortality of the animal. For dose progression, 1 to 8 slopes are given; based on

the test substance toxicity, the slope is chosen, and testing is initiated. Each slope dose starts from 0.175 mg/kg and ends with 5000 mg/kg. As the slope increases, the accuracy level of toxicity doses becomes more précised. In the absence of information on the slope of the substance to be tested, a dose progression factor of 3.2 is used. If the animal survives, the dose is increased by 3.2 times the original dose, and if the animal dies, the dose is decreased by 3.2 times the initial dose. This guideline of up and down procedure based on results, LD_{50} and confidence interval is estimated. It is the easiest method to apply to materials that produce death within 1-2 days. In case of delayed death, the method is not practically possible. Computer software is also available for the results of OECD guideline 425.

CONCLUSION

Acute oral toxicity study is significant before the efficacy study of the formulation. It evaluates the safe dose and toxic dose of the drug. Thus, it serves as the basis for further toxicity studies, i.e. sub-acute, sub-chronic and chronic toxicity studies. OECD guidelines 420, 423 and 425 are recommended for the acute oral toxicity study. The outcomes of OECD guidelines 420, 423 and 425 allow a substance to be ranked and classified according to the Globally Harmonised System for the hazard assessment of chemicals and provide LD_{50}. The procedure in OECD guideline 420 is short and easy to perform, and their results are based on a single step. OECD guideline 423 is a 2-step procedure, so the exact GHS category and LD_{50} cut-off values are obtained. OECD guideline 425 provides LD_{50} with a confidence interval but is a complicated procedure, so there are more chances of error occurrence. Thus, OECD guideline 423 is more precise, convenient, and often used for acute oral toxicity studies.

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Cite this article as:

Gothe Shivani Rameshwar, Pawade Uday Venkatrao, Nikam Ashwin Vithalrao, Anjankar Meghsham Pramodrao. OECD guidelines for acute oral toxicity studies: An overview. Int. J. Res. Ayurveda Pharm. 2023;14(4):137-140
DOI: http://dx.doi.org/10.7897/2277-4343.1404130

Source of support: Nil, Conflict of interest: None Declared