Description of the test procedure

Preparations
Healthy young adult rats are randomly selected and acclimatised to the laboratory conditions for at least 5 days prior to the test.

Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of solution in oil (e.g. corn oil) and then by possible solution in other vehicles. For non-aqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test. The maximum volume of liquid administered at one time should not exceed 1 ml/100 g body wt, except in the cases of aqueous solutions where 2 ml/100 g may be used. Variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels.

Experimental animals

Selection of species. The rat should be used. Commonly used laboratory strains should be employed. The weight variation in animals used in a test should not exceed ± 20% of the mean weight.

Number and sex. At least 10 animals (5 female and 5 male) should be used for each dose level which is investigated. The females should be nulliparous and non-pregnant.

Housing and feeding conditions. The temperature of the experimental animal room should be 22°C (± 3°) and the relative humidity 30–70%. Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g. morbidity, excitability) may indicate a need for individual caging. Where the lighting is artificial, the sequence should be 12 h light, 12 h dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Test conditions

Dose level. The dose level to be used in the test should be selected from one of the three levels listed in the criteria for classification, (Appendix Table 1) namely, 5, 50 or 500 mg/kg body wt. The initial dose level chosen should be that which is judged likely to produce evident toxicity but no mortality. Where no information is available upon which to make such a judgement, an initial 'sighting' study should normally be carried out. Where evident toxicity does not result from administration of the chosen dose level, the substance should be retested at the next higher dose level. The animals, however, should continue to be kept under observation until the observation period is complete. Where a severe toxic reaction requires animals to be removed from the study, the substance should be retested at the next lower dose level. Again, animals that do not need to be removed from the study should be kept under observation for the full observation period.

Appendix Table 1 Investigation of acute oral toxicity using a fixed dose procedure criteria for classification for labelling purposes

Test dose (mg/kg)	*Result*	*Action*
5	Less than 90% survival	Classify as *very toxic*
	90% or more survival; but evident toxicity	Classify as *toxic*
	90% or more survival; no evident toxicity	Retest at 50 mg/kg
50	Less than 90% survival	Classify as *toxic* Retest at 5 mg/kg if not already tested at that dosage
	90% or more survival; but evident toxicity	Classify as *harmful*
	90% or more survival; no evident toxicity	Retest at 500 mg/kg
500	Less than 90% survival *or* evident toxicity and no deaths	Classify as *harmful* Retest at 50 mg/kg if not already tested at that dose
	No evident toxicity	*Unclassified*

Observation period. Except where a test is prematurely terminated for animal welfare reasons, the observation period should be at least 14 d. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and may thus be extended when considered necessary. The time at which signs of toxicity appear and disappear must be recorded. If deaths occur, or if animals are humanely killed, the time of death should be noted.

Procedure
Animals should be fasted prior to substance administration by withholding food overnight. Following the period of fasting, the animals should be weighed and then the test substance administered in a single dose to animals by gavage using a stomach tube or a suitable intubation cannula. If a single dose is not possible, the dose may be given in smaller fractions