

LD 50 Toxicity Test On Animals

The Lethal Dose 50 Test Procedure

If there is one area where many scientists and animal rights campaigners are united it is in their condemnation of the LD50 test. LD stands for lethal dose and LD50 signifies the single dose needed to kill 50% of the animals used in the experiment. It is widely used to test the toxicity of household products, pesticides, cosmetics, drugs, weed killers and industrial products.

A common form of the test is by oral dosing using a tube inserted down the animal's throat. Other forms of dosing include injection, forced breathing of the vapour (LC50, lethal concentration 50%), and application of the substance to the animal's skin. Rabbits, rats, mice, birds and fish are the usual species with dogs and monkeys also used. In the "classical" or "formal" LD50 several dose levels are administered to equal groups of male and female animals. An average of 60 animals may be used, although this would only be for one species and one particular dosing method.

The test is allowed to proceed for 14 days, assuming the animals have not already died. Common signs of poisoning include "unusual vocalisation", tears, diarrhoea, discharge and bleeding from the eyes or mouth, and convulsions. No pain relief is given, yet the test must undoubtedly cause great suffering.

Humanely killing an animal during an LD50 test may invalidate the test results since, if left, the animal might have lingered on for the 14 days, or even possibly recovered, and therefore counted as a survivor. The more animals that survive, the less toxic the substance is judged to be, with the corresponding commercial advantage of a lower hazard classification,

Scientific Limitations LD 50 Test on Animals

The original purpose of the LD50 animal test, introduced by Trevan in 1927, was to measure the strength of drugs like Digitalis. Nowadays, such tests are rarely performed but the LD50 is still with us, and is now used as a crude index of acute toxicity, a purpose for which it was not designed.

Acute toxicity tests, of which the LD50 is a particularly common type are meant to provide information about:

1. Adverse effects of substances after a single dose.

2. Symptoms of overdose and the human lethal dose.
3. Selection of doses for the more prolonged animal tests.
4. Hazard classification of industrial chemicals.

Each of these areas will be considered in more detail to assess the value of information provided by the LD50 test on animals.

Adverse Effects of Substances after a Single Dose

The stated purpose of acute toxicity tests is to determine the adverse effects of substances after a single dose. But if this is the case, why have a death test like the LD50? When new medicines are given to human volunteers for the first time, only minute doses are given, working gradually upwards with any effects carefully monitored. Such studies involve careful clinical observation with regular blood, liver and kidney function tests so that risks are minimised for those taking part. Even if animals are to be used to identify the adverse effects of substances prior to clinical trials with volunteers and patients, there can be no scientific justification for a test like the LD50, where the animals are deliberately poisoned to death.

Another aspect is that enough of any substance, however harmless, will cause undesirable effects, and death may be caused by overpowering the animal's ability to cope with the sheer quantities given rather than by any particular poisonous action of the chemical. The kind of grotesque experiment in which huge quantities of harmless chemicals are given, thereby overloading one or more of the body's organs, and finally causing death, is totally unrelated to the human situation. But it is not only the high doses which make such experiments unreal. In similar situations, human beings might vomit to help remove the substance. However, rats cannot vomit. The stupidity of such tests has led to the suggestion that limit tests be used as an "alternative", that is, if the animals have not died after a certain, reasonably high dose, then the tests would be stopped.

Symptoms of Overdose and the Human Lethal Dose

It might be thought that LD50 results would be useful for emergency physicians in cases of accidental poisoning or intentional overdose. So it is particularly revealing to hear the views of Dr. Goulding, who established the first British National Poisons Information Service at Guy's Hospital in London.

“Whilst the data from animal studies..... provide some basic information of the mechanism of toxicity and relative toxicity, it cannot be assumed that this information will be entirely relevant for man.” “Experience gained from a careful assessment of patients suffering from acute overdose of drugs is potentially much more useful than that obtained from animal tests.”

An example is the painkiller, Paracetamol, which is frequently used in suicide attempts. This drug causes death in mice and hamsters by liver damage (LD50 250-400 mg/kg), but in rats the LD50 is considerably higher (1000 mg/kg) and even then it is hardly possible to see liver damage.

“How can the physician from such controversial data predict the response of human subjects?”

Species variation can also be a major problem when attempting to predict the human lethal dose. For example, the LD50 for Digitoxin in rats is 670 times that in cats, whilst for the antifungal substance Antimycin A, the LD50 in chickens is 30-80 times greater than in pigeons and mallards.

It is rarely possible to extrapolate from the LD50 in animals to the lethal dose in man.

It is only accidental human exposure which can give a reasonably reliable indication of the lethal dose and of the symptoms of overdose. This is emphasised by the complicating factors which often occur in overdose situations, such as alcohol abuse, disease, age and marked individual differences in susceptibility.

Whilst on the subject of estimating human lethal doses, it should be said that LD50 results cannot be used as a guide to the dose given to human volunteers in clinical trials, again because of the enormous differences which can occur between animals and man. For the safety of volunteers taking part, such trials must commence with minute amounts of the drug, whatever the preliminary tests have indicated.

Election of Doses for More Prolonged Animal Tests LD50

It might also be thought that LD50 values would help in the selection of dose levels for the more prolonged animal tests, taking several months, i.e. if a certain dose is fatal then less must be used for the subacute and chronic toxicity tests. The question is, how much less? In fact the LD50 is often a very poor guide in this respect since poisonous effects of repeated dosing cannot often be predicted from a test using a single dose, such as the LD50. An example is the corticosteroid hormone, Dexamethazone.

The LD50 in rats was found to be 120 mg/kg but on repeated administration, rats and dogs could not tolerate daily doses above 0.07 mg/kg, approximately 1700 times less than the LD50 value!

Hazard Classification of Industrial Chemicals Animal Research

Many industrial chemicals are subjected to LD50 determination and the information used to classify the substances according to their toxicity. Thus an oral LD50 in the rat of less than 25mg/kg would serve to classify the chemical as "very toxic", whilst an LD50 between 25 and 200 mg/kg would classify the substance as "toxic". Such information, so the argument goes, enables suitable precautions to be taken during transport, and in the workplace and environment. But how can sensible decisions be taken regarding human welfare on the basis of a test where results can vary enormously between the species. Classifying chemicals on the basis of LD50 tests could therefore be dangerously misleading.

Another factor is that worker exposure tends to be on a continuous basis and the likely effects of such exposure cannot be predicted by a single dose test, like the LD50.

If new chemicals are to be introduced, worker safety is best protected by ensuring exposure is kept to an absolute minimum, whatever preliminary animal or alternative tests might suggest. Additionally, workers should be closely monitored to give advance warning of any potentially dangerous effects.

Further Limitations Interlaboratory Variation Animal Testing

Having discussed the LD50 animal test in some detail, it can be appreciated that it has very little value. But not only is the LD50 dependent on species, it can also be influenced by many other factors including sex, age, diet, genetic strain, health, degree of starvation, method of dosing, temperature and humidity and even bedding material! It is customary for animals to be starved prior to testing, and this too can have a substantial effect. One example is the barbiturate Sodium Methohexiton. In mice allowed free access to food, the oral LD50 was 354 mg/kg. When food was withheld for 4-6 hours, the LD50 decreased to 162 mg/kg, whilst after 20 hours' food deprivation the LD50 was only 66 mg/kg.

The number of animals per cage can also affect results. The LD50 of the drug Isoproterenol decreased from 800 mg/kg to 50 mg/kg as a result of isolating the animals for three months.

The large number of variables which can affect test results shows that the LD50 is not an unchanging biological constant and the idea of using large numbers of animals to obtain more accurate figures is of no scientific value. In fact studies have shown that a chemical's LD50 value can vary from laboratory to laboratory by as much as 8-14 times, using the same species and the same method of dosing!

The Real Alternative to LD50 Test on Animals

For all the various, applications of acute toxicity testing, the LD50 test on animals is clearly of very little value, and could even be potentially dangerous. The test serves no medical or truly scientific purpose and should therefore be abandoned.

Why Does the LD50 Toxicity Test on Animals Continue?

If the LD50 is of such little value, why does it continue? The test is principally carried out for legislative purposes and has become part of a checklist.

In addition, the test may be of value to manufacturers, should they have to defend themselves against claims by consumers. And there are other financial reasons for the test to continue. In order to export their products, companies must fulfil the testing requirements of the countries involved, and these are likely to include LD50 tests.

Several countries, including America and the UK, have a net favourable balance of trade in pharmaceuticals – sufficient reason for governments to defend the status quo.

The LD50 animal test has continued then, not to fulfil any real scientific need but for commercial and bureaucratic reasons.

Conclusions Toxicity LD50 Test on Animals

1. It has been acknowledged that LD50s must cause appreciable pain and considerable suffering to a proportion of the animals subjected to them.
2. The LD50 is a particularly common form of acute toxicity test, but for all the various applications of such tests, the LD50 is not only of very little value but even potentially dangerous.
3. The test has become a ritual mass slaughter and serves no useful medical or truly scientific purpose. It should therefore be abandoned and

the International Association Against Painful Experiments on Animals calls for its complete prohibition.