throughout a specified time period up to 14 days. All deaths and some symptoms are recorded. Common signs of poisoning include unusual vocalization, tears, diarrhea, discharge and bleeding from the eyes or mouth, and convulsions.[5] All surviving animals are killed, autopsied, and analyzed to determine which organs or systems have been affected and in what manner. The LD-50 is derived from the numbers of deaths occurring from each of the doses given.

Even with large numbers of animals, there are considerable variations in test results due to the influence of such factors as animal species/strain, age and sex, diet, food deprivation prior to testing, temperature, caging conditions, season, experimental procedures, etc. But even if all of these variables could be controlled, there is still the basic problem of extrapolation to humans. No matter how specific the LD-50 value may be, the test cannot determine precisely what effect the test substance will have on a human being. It should also be pointed out that LD-50 determinations concerning pharmacologically inert chemicals, such as food additives, require excessively high doses. Animals are often killed by the sheer mass of non-toxic substances administered.

As previously noted, the LD-50 gives no information at all on the long-term effects of the materials tested. Yet long-term exposure may constitute the greatest threat of all to human beings.

Presently, there are several tests which can approximate the LD-50. These involve fewer animals and their precision and reproducibility are sufficient for most toxicity testing purposes.

Careful observation of the signs of toxicity, determination of the target organs of acute toxicity, and estimation of the likelihood of recovery from acute poisoning, yield data much more reliable than knowledge of a precise LD-50 value. Using modern clinical, biochemical, and histological laboratory methods, approximate LD-50 values can be supplied using very few animals. These procedures require that a range of doses be given, using only two animals per dose, until the first deaths occur. All signs of disorder are noted. The test provides a minimum lethal dose value, the maximum tolerated single dose, and an approximate LD-50. These techniques are already in general use in toxicity studies with large animals. This approach, already proven successful, could be used to reduce drastically the numbers of rodents and other small animals that otherwise would be "sacrificed."

[5]Paget, G.D., Ed., *Methods in Toxicology* (Blackwell Scientific Publications, United Kingdom, 1970).

Another alternative to the LD-50 is the "limit test" for non-toxic chemicals, sometimes accepted by regulatory authorities, which can also be expanded. A single, pre-selected non-lethal dose (5 g/Kg orally and 2 g/Kg parenterally) is administered to a small number of animals; if no deaths or ill effects occur, no further testing is required. Advantageous for testing cosmetics and food additives, etc., it avoids killing an animal simply by the administration of large quantities of the test substance.

A mathematical model is also being developed by Kurt Enslein to study the LD-50 (see page 6 of this report).

Various trade associations, including the Pharmaceutical Manufacturers Association (PMA), have urged that changes be made in some of the regulations requiring the LD-50 test. These groups have determined, in independent studies, that carefully controlled acute experiments produce more relevant and meaningful data than LD-50 values alone. They recommend:

1) Limiting precise LD-50 testing to those cases where it is scientifically necessary for the comparison of potencies of different batches of the same chemical, or different chemicals of similar structure and/or activity.
2) Encouraging use of acute safety studies that yield a maximum amount of data from using a minimum number of animals. An approximate LD-50 value or range definition of toxic dosage usually represents adequate information on the acute toxicity of drugs.
3) Persuading the regulatory agencies of all countries that a precise LD-50 determination is not necessary. Since drugs are distributed worldwide, the present protocols are designed to satisfy the requirements of the most demanding country.

The tendency to assume that acute toxicity can be adequately defined only by the LD-50 test must be countered by information on the value of approximate LD-50 determinations. The standard LD-50 test is simply a numeric index of lethality; while it may have some utility as a reference point, it is not usually necessary to perform a full LD-50 study to determine the acute toxicity of a given compound. Furthermore, LD-50 test results can be influenced by experimental variables that are not controlled by the protocol.

Many scientists concur in this view. In 1969, Baker stated that acute studies "are of little use and are ex-