Inadequacy of the LD50 Test

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"[The LD50] is now an anachronism….I do not think the LD50 test provides much useful information about the health hazards to humans."

— David Rall, Ph.D., former Director
National Toxicology Program

The Lethal Dose 50 (LD50) test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50 percent of the test subjects within a set time frame. Typically, administration of the test substance is via a tube inserted down the esophagus into the stomach. Other routes of administration include inhalation and applying the substance to the animals' skin. Several dose levels of a chemical are given until the dose lethal to half of the test population is attained. The test is typically allowed to proceed for 14 days, at which time all the animals who have not died from the test substance are killed.

Animals who have not died within the test period may be sick or near death. The LD50 provides no information on what system failure led to the death of the animals. Some deaths may be due to the quantity of the test substance causing gastric rupture or other morbidity unrelated to the toxicity of the test substance.

The designers of the LD50 test in 1927 acknowledged its serious inadequacies, intending it only for certain narrow medical purposes.[1] Nevertheless, use of the LD50 test has become widespread as a general measurement of chemical toxicity. The LD50 has been challenged for decades as both unreliable and uninformative.

The LD50 Is Highly Unreliable

Small changes in test conditions can produce wildly varying outcomes. It has been well documented that species,[2] strain,[3] and age[4] have marked effects on LD50 results, as do weight,[5] sex,[4] health,[6] diet,[4] whether the animals are deprived of food before the test,[7] the method by which the chemical is administered,[8] ambient temperature,[9,10] and housing conditions of the animals.[6,11] These factors lead to LD50 measurements that differ by orders of magnitude. It is likely that other factors, including humidity, weather, noise, the light-dark cycle, and the dexterity of the laboratory personnel, can also affect the outcome of the test. A study arranged by the Commission of the European Communities found that LD50 values, based on tests of the same substances performed in different laboratories, differed by as much as a factor of 12. A second trial which attempted to standardize conditions across laboratories still yielded results differing by as much as a factor of eight from one laboratory to the next.[12] It is clear that the quantity measured by the LD50 test is not a biological constant, and that the value therefore has little significance in assessing toxicity.

The LD50 Has Little Relevance for Human Toxicity

Even if the LD50 were reliable, the information it provides is of little use to humans for several reasons.

- Species-to-species differences in sensitivity give the LD50 test little predictive capability for assessing toxicity in humans (see chart). Acetaminophen, for example, is fatal to mice at 250-400 mg/kg due to liver necrosis, while the LD50 for rats is about 1,000 mg/kg with little evidence of liver damage.[13] With such profound differences between mice and rats, extrapolation to humans has little meaning. Indeed, a comparison of the toxicities of various chemicals for humans and animals found large differences to be typical.[14] A recent multi-center study found that even under the most standardized conditions, the correlation between animal LD50 values and acute toxicity in humans was only 63 percent.[15] As an Institute for Toxicology scientist has commented, "[E]ven if the LD50 could be measured exactly and reproducibly, the knowledge of its precise numerical value would barely be of practical importance, because extrapolation from the experimental animals to man is hardly possible."[16]
- The LD50 measures only lethality, ignoring other adverse effects which often correlate poorly with mortality. Thus a chemical can have extremely harmful but nonlethal effects at doses far short of the LD50 dosage.
- Pretreatment with small doses of some chemicals (e.g., cadmium chloride) raises the LD50 level, and other substances

are lethal at 1/100th the LD50 value when taken daily.

• For pharmacologically inert compounds, the LD50 may measure properties of no significance to human exposure. For example, inosic acid, a flavor enhancer added to food in trace amounts, was found lethal at doses of 20 g/kg, not from true toxicity, but by raising stomach acidity high enough to cause corrosion of the gastrointestinal lining. An equivalent dose in humans would flavor six tons of food.[17]

• Roughly 80 to 90 percent of poisonings involve children under five years of age, who commonly react very differently from adults to chemical substances. A study comparing toxicity in newborn and adult animals found large variations due to species-specific developmental patterns that cannot be readily extrapolated to human infants.[4]

• In practice, 50 percent of adult overdoses and 90 percent of narcotic overdoses involve mixtures of drugs, and often the substances ingested are not known. The LD50 test does not account for drug interactions, and is therefore of little use in such cases.[18]

The serious inadequacies of the LD50 test leave it "only marginally informative, toxicologically inadequate, and misleading."[19]

The LD50 Is a Poor Choice of Test

Modifications, such as the up-down and limit tests, are simply refinements of the classic LD50 test, and suffer from the same deficiencies. However, *in vitro* methods are available that produce highly reliable results and provide more predictive information about the effects of chemicals on human beings. As David Rall, Ph.D., then-director of the National Toxicology Program (NTP), wrote in March 1983, the LD50 "is now an anachronism….I do not think the LD50 test provides much useful information about the health hazards to humans."[20] The LD50 is a highly unsatisfactory measure of toxicity in humans.

Comparison of the LD50 in Rats and Mice

(NIOSH/Registry of Toxic Effects of Chemical Substances)

Chemical	*Rat mg/kg*	*Mouse mg/kg*	*Ratio*
Carbon tetrachloride	2350	8260	.28
Dextropropoxyphene HCl	84	225	.37
Dichloromethane	1600	873	1.8
Diphenylhydantoin	1640	150	10.9
Ethanol	7060	3450	2.0
Mercury (II) chloride	1	6	.17
Nicotine	50	3	16.7
Paracetamol	2400	340	7.0
Sodium oxalate	11,200	5100	2.2
Thioridazine HCl	995	385	2.6

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