Editorials

The LD50 — The Beginning of the End

Andrew N. Rowan

The Basis of the Argument

Measurement is very important in science. Early lessons in the science classroom involve teaching students to measure lengths, volumes, weights, specific gravities and anything else within the mental and economic compass of the teacher. At the same time, the question of *significance* is drummed into the students' heads. Thus, if one has a meter-rule which is subdivided into centimeters (but not millimeters), one is taught that the measurement of its length to one decimal point (for example, 10.3 cm) is acceptable, but that the addition of any more figures (for example, 10.325 cm) is mere braggadocio. The eye can only make a rough guess at the subdivision between the centimeter divisions, and adding more figures after the decimal point does not improve the accuracy of the estimate.

However, adding more numbers, without increasing the accuracy of the measurement, is precisely what is being attempted when the LD50 is used as a measure of the acute toxicity of chemicals. (The LD50 is the amount of a substance which, if administered in a single dose to a target group of animals, will kill 50 percent of them). Normally, 50 to 200 animals are used to estimate the LD50 *and* provide its standard deviation from the mean. For some reason, regulators and some toxicologists appear to believe that an LD50 with its fiducial limits is more accurate and more relevant than a *rough estimate* of the acute toxicity, an estimate that can be obtained by using as few as six animals (rather than the 50-200 animals needed for an LD50).

The point at issue, therefore, is simply this: Animal welfare groups and many toxicologists want to see the LD50 (performed on 50 or more animals) replaced within the next year by a rough estimate of acute toxicity. The regulatory authorities have so far resisted making the necessary changes.

History

In 1927, J.W. Trevan published his classic report on toxicity determination, in which he asserted that the median lethal dose (or LD50), done in a large (50-200) sample of animals, provided the most accurate index of a chemical's toxicity (*Proc Roy Acad Soc 101B*:483-514). He was, however, concerned mainly with the accurate standardization, by biological methods, of those drugs that are not available in a chemically pure form. For example, each new batch of such important drugs as digitalis extract, insulin, and diphtheria toxin had to be accurately standardized since the margin of safety between therapeutic and toxic doses is so small. Even today, the *U.S. Pharmacopoeia* requires a bioassay standardization of powdered digitalis that involves comparing the lethal dose in pigeons against a reference standard.

However, the number of LD50 determinations used to standardize potent biological therapeutics now represents only a small proportion of the LD50 tests conducted annually. Most LD50 testing is done to provide a figure for the toxicity

of other classes of chemicals. But somehow the LD50 figure has gained a totally undeserved position as *the* toxicological reference standard; it seems to be regarded in nearly the same light as such physical constants as melting point and specific gravity. But as Trevan and his colleagues recognized, the LD50 of a substance is not a fixed value; it varies according to many extraneous factors, sometimes by substantial amounts (see Tables 1 and 2).

In the last 15 years, however, the use of the LD50 as a toxicological standard has come in for increasing criticism among toxicologists (see *Arch Toxicol 47*: 77-99, 1981). It is not that they deny the need for some sort of rough numerical estimate of acute toxicity in a mammalian species. Rather, they deny the utility of the *precise statistical* figure that is provided by the usual LD50 test. It is most important that this point be clearly recognized. The immediate argument over the LD50 is not that we do not need acute toxicity data, but that we can get the kind of data we need from small-scale tests in a few animals. We do not need to kill as many animals as we do *merely* to provide statistical precision.

Protest Against the LD50

In the last decade, animal welfare criticism of the LD50 test has become increasingly vocal and sophisticated. In England, such criticism prompted a relatively unusual initiative from the Home Secretary. In 1977, he asked the Advisory Committee to the Cruelty to Animals Act, 1876, to review the extent of the use of the LD50 test, as well as the scientific necessity and justification for the test in its various applications. The Advisory Committee listened to extensive evidence from animal welfare critics and the scientific community. Interestingly, the scientific and regulatory groups, while more restrained in tone, were often just as critical of the LD50 test as the animal welfare groups.

The Association of the British Pharmaceutical Industry concluded that: "estimation of LD50 is not an essential requirement to ensure the safety of all new drugs. Adequate information regarding the acute toxicity, including the acute lethality, of new drugs can often be obtained by the use of smaller numbers of animals than are conventionally used in LD50 determinations." The Chemical Industries Association proposed that (1) regulatory agencies be discouraged from demanding precise LD50 figures; (2) emphasis be placed on the qualitative data obtainable from small-scale acute toxicity studies; and (3) no animal should be administered a quantity greater than 5 g (or 5 ml) of a substance per kg of body weight (the so-called Limit test). The Scottish Home and Health Department noted that "there is no case to be made for requiring LD50 tests to provide a value with small fiducial limits. An approximate estimate suffices."

By contrast, the Medical Research Council (MRC), after explaining that precise data on acute toxicity were not really necessary, concluded that "the LD50 test is the only reliable measure of acute toxicity and yields a result with the least possible expenditure of life." However, they followed this assertion with a statement that only a simple test, using a small number of animals, should be done to assess the *order of magnitude* of a chemical's toxicity. Clearly, when the MRC talked of the need for an LD50, they really meant that what we need to perform in most cases is a small-scale acute toxicity test.

Unfortunately, the MRC was not the only group to confuse the notion of small-scale acute toxicity testing with the LD50 test. When the Home Office report finally appeared in 1979, their first recommendation was that "LD50 tests should be allowed to continue." Although they qualified this recommendation by

advising that only a small numbers of animals need be used, the harm had been done: A government enquiry had found that LD50 tests needed to continue. I cannot say that I, personally, found the Committee's findings particularly surprising. When I gave testimony to the Committee (on behalf of FRAME—for whom I was working at the time), one of the expert advisors was almost plaintive in defending the toxicologist's need for a baseline figure for acute toxicity (*i.e.*, the LD50) and the other did not appear to accept the distinction between small-scale acute toxicity testing and the full LD50.

Recent Developments

Despite the setback presented by the 1979 report from the British Home Office, there are now some encouraging signs that an unlikely alliance of animal welfare and industrial organizations may prevail upon regulatory bodies and effect a revolution in acute toxicity testing. For example, if regulatory bodies would agree to prohibit the submission of LD50 figures except in those few cases where scientific justification can be provided for an LD50 determination, we would reduce the number of animals used in determining lethal doses by about 80-90 percent. Numerically, this would probably amount to 2-4 million animal lives saved every year. What events have occurred to change the climate of opinion since 1979?

First, an international coalition of animal welfare groups has been formed with the specific aim of abolishing the LD50 test. A similar coalition against the Draize test was very successful (see *Int J Stud Anim Prob* 3:94-97), and there is every reason to hope for similar success if a concerted campaign can be mounted over the next year. The immediate goal will be to get the regulatory agencies to switch from tacit or explicit requirements for LD50 data to an explicit prohibition on the submission of LD50 data, unless accompanied by scientific justification.

Second, on October 21, 1982, the Pharmaceutical Manufacturers Association (U.S.) called for a revision of government regulations so that fewer animals are used in drug safety evaluation. They specifically noted that "the classical LD50 test which utilizes many animals to determine an LD50 value with mathematical precision lacks justification..." They proposed that: (1) the *precise* determination of an LD50 should be limited to those rare cases where it is necessary; (2) an approximate lethal dose plus qualitative data usually represents adequate information on the acute toxicity of drugs; and (3) there should be an international effort to reach agreement among regulatory agencies that, for drugs, a precise LD50 determination is not necessary.

Third, at a number of recent scientific meetings, the overwhelming consensus has been that the LD50 is unnecessarily precise—qualitative and semi-quantitative data from small-scale acute toxicity tests is usually adequate. For example, at a FRAME conference, pharmaceutical company staff in the audience voted to abolish the LD50 test by 20 to 1 (*New Scientist*, November 4, 1982, p. 275). At a conference that specifically addressed the LD50 test in Sweden (September, 1981), a clinical toxicologist from the Karolinska Poison Information Center stated that the numerical information provided by an animal LD50 is virtually useless. Other scientific meetings on the use of animals in acute toxicity testing are planned. The indications are that these meetings will confirm the uselessness of precise LD50 data. All this activity on the part of scientists, combined with animal welfare protests, should escalate the pressure to the point that regulatory bodies are forced to take action.

Conclusion

A reassessment of the need for LD50 figures is long overdue. Bureaucrats may

not be comfortable with approximate lethal dose figures, but there are clearly few cases where LD50 determinations amount to anything more than pseudoscientific nonsense. LD50 testing continues, not because it receives broad endorsement, but because nobody feels sufficiently secure to take the decisive action that is necessary to eradicate 40 years of thoughtless tradition. Since death by poisoning cannot be particularly pleasant, regulatory agencies that are serious about animal welfare issues ought to begin to take steps to abolish unnecessary LD50 testing, especially since the scientific verdict against it is already in.

TABLE 1 Human Acute Lethal Doses and Animal LD50's (Oral)

	Human LDLo (mg/kg)	Animal LD50			
		Rat	Mouse	Rabbit	Dog
Amytal	43	560	—	575	—
Boric Acid	640	2660	3450	—	—
Caffeine	192	192	620	—	—
Carbofuran	11	5	2	—	—
Lindane	840	125	—	130	120
Fenflurazole	—	238	1600	28	—
Cycloheximide	—	3	133	—	65

Compiled from *CRC Handbook of Analytical Toxicology* and the NIOSH *Registry of Toxic Effects of Chemical Substances*

TABLE 2 Range of LD50 Values for Five Compounds Tested Under Similar Conditions in 65 Different European Toxicology Laboratories

Compound	LD50 Range (mg/kg)	
	Laboratories That Used Their Own Protocol	Laboratories That Used The Standard Protocol
PCP	46-522	74-2328
Na Salicylate	800-4150	930-2328
Aniline	350-1280	479-1169
Acetanilide	805-5420	723-3060
Cadmium Chloride	70-513	105-482

Compiled from *J Assoc Off Anal Chem* 62:864-873, 1979, and *Arch Toxicol* 47:77-98, 1981