BIO-X TECHNO

Good Earth Great Chemistry

WHAT'S LD_{50}

BXT - THE COMPANY

BIO-X TECHNO Sdn Bhd (BXT) was established with Bio-X Global (BXG) Pte Ltd of Singapore to research, develop, manufacture, market and globally distribute, a range of new-age "safe" pest.control and bio.growth "promoters".

Together with Bio-X Global (BXG) , we are a socially responsible organization, task with the application of biotechnology to develop environmentally-friendly crop protection and pest control products and services. We develop, manufacture and market plant growth promoters and safe insecticides based on organic natural plant extracts such as pine and neem.

We offer the best natural solutions that modern science can offer to help protect our ecology and the environment ... for planet earth!

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ENVIRONMENTAL POLICY

It is our policy at BXT that research, manufacturing and marketing activities be carried out in a manner which demonstrates responsibility to the environment and care for our earth and its inhabitants.

We are committed to :-

- Continually research and develop products for agricultural and public health applications that are not only environmentally friendly but also ecology balanced;
- Comply with all relevant environmental legislation and regulations;
- Prevent pollution in all activities and operations;
- Work with the community and government agencies to enhance environmental awareness and protection; and
- Strive towards continuous improvements in "environmental performance".

BXT POISONS BOARD LICENSE

Malaysian Ministry of Agriculture
No. Pendaftaran : LRMP. R1/9233 for BV 20EW
No. Pendaftaran: LRMP. R1/9234 for BX Q6

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WHAT IS LD50?

LD stands for "Lethal Dose". LD50 is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD50 is one way to measure the short-term poisoning potential (acute toxicity) of a material.

Toxicologists can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g. milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD50 can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common., a high-efficacy and extremely-safe insecticide with proprietary blends of certain botanical extracts as carrier and synergist.

What does LC50 mean?

LC stands for "Lethal Concentration". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water.

For inhalation experiments, the concentration of the chemical in air that kills 50% of the test animals in a given time (usually 4 hours) is the LC50 value.

Why study LD50?

Chemicals can have a wide range of effects on our health. Depending on how the chemical will be used, many kinds of toxicity tests may be required.

Since different chemicals cause different toxic effects, comparing the toxicity of one with another is hard. We could measure the amount of a chemical that causes kidney damage, for example, but not all chemicals will damage the kidney. We could say that nerve damage is observed when 10 grams of chemical A is administered, and kidney damage is observed when 10 grams of chemical B is administered. However, this information does not tell us if A or B is more toxic because we do not know which damage is more critical or harmful.

Therefore, to compare the toxic potency or intensity of different chemicals, researchers must measure the same effect. One way is to carry out lethality testing (the LD50 tests) by measuring how much of a chemical is required to cause death. This type of test is also referred to as a "quantal" test because it is measures an effect that "occurs" or "does not occur".

Who invented the idea of an LD50?

In 1927, J.W. Trevan attempted to find a way to estimate the relative poisoning potency of drugs and medicines used at that time. He developed the LD50 test because the use of death as a "target" allows for comparisons between chemicals that poison the body in very different ways. Since Trevan's early work, other scientists have developed different approaches for more direct, faster methods of obtaining the LD50.

NEW AGE "INSECTICIDE"

What are some other toxicity dose terms in common usage?

- LD01 - Lethal dose for 1% of the animal test population;
- LD100 - Lethal dose for 100% of the animal test population;
- LDLO - The lowest dose causing lethality; and
- TDLO - The lowest dose causing a toxic effect.

Why are LD50 and LC50 values a measure of acute toxicity?

Acute toxicity is the ability of a chemical to cause ill effects relatively soon after one oral administration or a 4-hour exposure to a chemical in air. "Relatively soon" is usually defined as a period of minutes, hours (up to 24) or days (up to about 2 weeks) but rarely longer.

How are LD50 /L C50 tests done?

In nearly all cases, LD50 tests are performed using a pure form of the chemical. Mixtures are rarely studied. The chemical may be given to the animals :-

- by mouth (oral);
- by applying on the skin (dermal); or
- by injection at sites such as the blood veins (i.v.- intravenous), muscles (i.m. - intramuscular) or into the abdominal cavity (i.p. - intraperitoneal).

The LD50 value obtained at the end of the experiment is identified as the LD50 (oral), LD50 (skin), LD50 (i.v.), etc., as appropriate. Researchers can do the test with any animal species but they use rats or mice most often. Other species include dogs, hamsters, cats, guinea-pigs, rabbits, and monkeys. In each case, the LD50 value is expressed as the weight of chemical administered per kilogram body weight of the animal and it states the test animal used and route of exposure or administration; e.g., LD50 (oral, rat) - 5 mg/kg, LD50 (skin, rabbit) - 5 g/kg. So, the example "LD50 (oral, rat) 5 mg/kg" means that 5 milligrams of that chemical for every 1 kilogram body weight of the rat, when administered in one dose by mouth, causes the death of 50% of the test group.

If the lethal effects from breathing a compound are to be tested, the chemical (usually a gas or vapor) is first mixed in a known concentration in a special air chamber where the test animals will be placed. This concentration is usually quoted as parts per million (ppm) or milligrams per cubic meter (mg/m3). In these experiments, the concentration that kills 50% of the animals is called an LC50 (Lethal Concentration 50) rather than an LD50. When an LC50 value is reported, it should also state the kind of test animal studied and the duration of the exposure, e.g., LC50 (rat) - 1000 ppm/4 hr or LC50 (mouse) - 5mg/m3/2hr.

Which LD50 information is the most important for occupational health and safety purposes?

Inhalation and skin absorption are the most common routes by which workplace chemicals enter the body. Thus, the most relevant from the occupational exposure viewpoint are the inhalation and skin application tests. Despite this fact, the most frequently performed lethality study is the oral LD50. This difference occurs because giving chemicals to animals by mouth is much easier and less expensive than other techniques.

NEW AGE ... NON-TOXIC?
INSECTICIDE ... SAFE?

However, the results of oral studies are important for drugs, food poisonings, and accidental domestic poisonings. Oral occupational poisonings might occur by contamination of food or cigarettes from unwashed hands, and by accidental swallowing.

How do I compare one LD50 value to another and what does it mean to humans?

In general, the smaller the LD50 value, the more toxic the chemical is. The opposite is also true : the larger the LD50 value, the lower the toxicity.

The LD50 gives a measure of the immediate or acute toxicity of a chemical in the strain, sex, and age group of a particular animal species being tested. Changing any of these variables (e.g., type animal or age) could result in finding a different LD50 value. The LD50 test was neither designed nor intended to give information on long-term exposure effects of a chemical.

Once you have an LD50 value, it can be compared to other values by using a toxicity scale. Confusion sometimes occurs because several different toxicity scales are in use. The two most common scales used are the "Hodge and Sterner Scale" and the "Gosselin, Smith and Hodge Scale". These tables differ in both the numerical rating given to each class and the terms used to describe each class. For example, a chemical with an oral LD50 value of 2 mg/kg, would be rated as "1" and "highly toxic" according to the Hodge and Sterner Scale (refer to Table 1.0) but rated as "6" and "super toxic" according to the Gosselin, Smith and Hodge Scale (refer to Table 2.0). It is important to reference the scale you used when classifying a compound.

It is also important to know that the actual LD50 value may be different for a given chemical depending on the route of exposure (e.g., oral, dermal, inhalation). For example, some LD50 for dichlorvos, an insecticide commonly used in household pesticide strips, are listed below :-

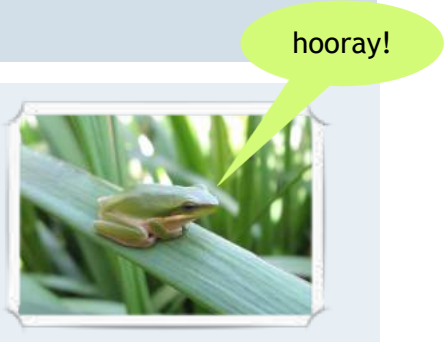
- Oral LD50 (rat): 56 mg/kg
- Dermal LD50 (rat): 75 mg/kg
- Intraperitoneal LD50 (rat) : 15 mg/kg
- Inhalation LC50 (rat) : 1.7 ppm (15 mg/m3); 4-hour exposure
- Oral LD50 (rabbit) : 10 mg/kg
- Oral LD50 (pigeon:) : 23.7 mg/kg
- Oral LD50 (rat) : 56 mg/kg
- Oral (mouse) : 61 mg/kg
- Oral (dog) : 100 mg/kg
- Oral (pig) : 157 mg/kg

Differences in the LD50 toxicity ratings reflect the different routes of exposure. The toxicity rating can be different for different animals. The data above show that dichlorvos is much less toxic by ingestion in pigs or dogs than in rats. Using Table 1, dichlorvos is moderately toxic when swallowed (oral LD50) and extremely toxic when breathed (inhalation LC50) in the rat. Using Table 2, dichlorvos is considered very toxic when swallowed (oral LD50) by a rat

Can animal LD50 data be applied to man?

In general, if the immediate toxicity is similar in all of the different animals tested, the degree of immediate toxicity will probably be similar for humans. When the LD50 values are different for various animal species, one has to make approximations and assumptions when estimating the probable lethal dose for man. Tables 1 and 2 have a column for estimated lethal doses in man. Special calculations are used when translating animal LD50 values to possible lethal dose values for humans. Safety factors of 10,000 or 1,000 are usually included in such calculations to allow for the variability between individuals and how they react to a chemical, and for the uncertainties of experiment test results.

IMAGINE … AN "INSECTICIDE" THAT IS BOTH ECOLOGICALLY-BALANCED, ENVIRONMENTALLY-FRIENDLY! AND NON-POISONOUS



How should an LD50 value be used?

The LD50 can be used :-

- As an aid in developing emergency procedures in case of a major spill or accident;
- To help develop guidelines for the use of appropriate safety clothing and equipment. For example, if the dermal LD50 value for a chemical is rated as extremely toxic, it is important to protect the skin with clothing, gloves (etc.) made of the right chemical-resistant material before handling. Alternatively, if a chemical has an inhalation LC50 value which indicates that it is relatively harmless, respiratory protective equipment may not be necessary (as long as the oxygen concentration in the air is in the normal range - around 18%);
- For the development of transportation regulations;
- As an aid in establishing occupational exposure limits; and
- As a part of the information in Material Safety Data Sheets. Remember, the LD50 is only a ball park figure so that lethal toxicity can be compared. It says nothing about levels at which other acute toxic, but non-lethal, effects might occur.

The LD50 is only one source of toxicity information. For a more thorough picture of the immediate or acute toxicity of a chemical, additional information should be considered such as the lowest dose that causes a toxic effect (TDLO), the rate of recovery from a toxic effect, and the possibility that exposure to some mixtures may result in increasing the toxic effect

Where can I find LD50 and LC50 values?

The largest, single collection of LD50 and LC50 values is in the database Registry of Toxic Effects of Chemical Substances (RTECS) that is available by subscription on CD-ROM and on the Internet. Two other databases available from CCOHS, CHEMINFO and the Hazardous Substances Data Bank® (HSDB). Both of these are on the CHEMpendium CD-ROMlivepage.apple.com; CHEMINFO is also accessible on the Internet

Are there weaknesses in LD50 values?

Any testing method has strengths and weaknesses. However, LD50 is being phased out in some jurisdictions in favor of tests such as the Fixed Dose Procedure, however the concept, and calculation of the median lethal dose for comparison purposes, is still widely used.

As a measure of toxicity, LD50 is somewhat unreliable and results may vary greatly between testing facilities due to factors such as the genetic characteristics of the sample, population, animal species tested, environmental factors and mode of administration ... so the credibility of the testing lab and/or authority greatly enhances the reliability of the test results.

Another weakness is that it measures acute toxicity only (as opposed to chronic toxicity at lower doses), and does not take into account toxic effects that do not result in death but are nonetheless serious (e.g. brain damage). There can be wide variability between species as well; what is relatively safe for rats may very well be extremely toxic for humans, and vice versa. In other words, a relatively high LD50 does not necessarily mean a substance is harmless, but a very low one is always a cause for concern. The term semi-lethal dose is occasionally used with the same meaning, particularly in translations from non-English-language texts, but can also refer to a sublethal dose; because of this ambiguity, it is usually avoided.

LD50 Conventions

The LD50 is usually expressed as the mass of substance administered per unit mass of test subject, such as grams of substance per kilogram of body mass. Stating it this way allows the relative toxicity of different substances to be compared, and normalizes for the variation in the size of the animals exposed (although toxicity does not always scale simply with body mass). Typically, the LD50 of a substance is given in milligrams per kilogram of body weight. In the case of some neurotoxins such as batrachotoxin, one of the most deadly toxins known, the LD50 may be more conveniently expressed as micrograms per kilogram (µg/kg) of body mass.

The choice of 50% lethality as a benchmark avoids the potential for ambiguity of making measurements in the extremes, and reduces the amount of testing required. However, this also means that LD50 is not the lethal dose for all subjects; some may be killed by much less, while others survive doses far higher than the LD50. Measures such as LD1 and LD99 (dosage required to kill 1% or 99% respectively of the test population) are occasionally used for specific purposes. Lethal dosage often varies depending on the method of administration; for instance, many substances are less toxic when administered orally than when intravenously administered. For this reason, LD50 figures are often qualified with the mode of administration, e.g. "LD50 i.v."

Table 1 : Toxicity Classes - Hodge and Sterner Scale

		Routes of Administration			
		Oral	Inhalation	Dermal	
Toxicity Rating	Commonly Used Term	(single dose to rats) mg/kg	(exposure of rats for 4 hours) ppm	(single application to skin of rabbits) mg/kg	Probable Lethal Dose for Man
1	Extremely Toxic	1 or less	10 or less	5 or less	1 grain (a taste, a drop)
2	Highly Toxic	1-50	10-100	5-43	4 ml (1 tsp)
3	Moderately Toxic	50-500	100-1000	44-340	30 ml (1 fl. oz.)
4	Slightly Toxic	500-5000	1000-10,000	350-2810	600 ml (1 pint)
5	Practically Non-Toxic	5000-15,000	10,000-100,000	2820-22,590	1L (or 1 quart)
6	Relatively Harmless	15,000 or more	100,000	22,600 or more	1L (or 1 quart)

The related quantities LD50/30 or an LD50/60 are used to refer to a dose that without treatment will be lethal to 50% of the population within (respectively) 30 or 60 days. These measures are used more commonly within Radiatin Health Physics as survival beyond 60 days usually results in recovery.

Table 2 : Toxicity Classes - Gosselin, Smith and Hodge

Probable Oral Lethal Dose (Human)		
Toxicity Rating or Class	Dose	For 70-kg Person (150 lbs)
6 Super Toxic	Less than 5 mg/kg	1 grain (a taste - less than 7 drops)
5 Extremely Toxic	5-50 mg/kg	4 ml (between 7 drops and 1 tsp)
4 Very Toxic	50-500 mg/kg	30 ml (between 1 tsp and 1 fl ounce)
3 Moderately Toxic	0.5-5 g/kg	30-600 ml (between 1 fl oz and 1 pint)
2 Slightly Toxic	5-15 g/kg	600-1200 ml (between 1 pint to 1 quart)
1 Practically Non-Toxic	Above 15 g/kg	More than 1200 ml (more than 1 quart)

A comparable measurement is LCt50 which relates to lethal dosage from exposure, where C is concentration and t is time. It is often expressed in terms of mg-min/m³. ICt50 is the dose which will cause incapacitation rather than death. These measures are commonly used to indicate the comparative efficacy of chemical warfare agents, and dosages are typically qualified by rates of breathing (e.g., resting = 10 l/min) for inhalation, or degree of clothing for skin penetration. The concept of Ct was first proposed by Fritz Haber, and is sometimes referred to as Haber's Law, which assumes that exposure to 1 minute of 100 mg/m³ is equivalent to 10 minutes of 10 mg/m³ (1 × 100 = 100, as does 10 × 10 = 100).

Some chemicals, such as hydrogen cyanide are rapidly detoxified by the human body, and do not follow Haber's Law. So in these cases the lethal concentration may be given simply as LC50 and qualified by a duration of exposure (e.g. 10 minutes). The Material Safety Data Sheets for toxic substances frequently use this form of the term even if the substance does follow Haber's Law.

For disease-causing organisms, there is also a measure known as the median infective dose and dosage. The median infective dose (ID50) is the number of organisms received by a person or test animal qualified by the route of administration (e.g., 1,200 org/man per oral). Because of the difficulties in counting actual organisms in a dose, infective doses may be expressed in terms of biological assay, such as the number of LD50's to some test animal. In biological warfare infective dosage is the number of infective doses per minute for a cubic meter (e.g., ICt50 is 100 medium doses - min/m^3).

LD50 Animal Rights Concerns

Animal-rights and animal-welfare groups, such as Animal Rights International, have campaigned against LD50 testing on animals in particular as, in the case of some substances, causing the animals to die slow, painful deaths. Several countries have taken steps to ban the oral LD50, and the OECD abolished the requirement for the oral test in 2001 (see Test Guideline 401, Trends in Pharmacological Sciences Vol 22, February 22/01').

LD50 still remains popular, despite its general weakness in providing a useful measure of toxicity.

Table 3 :

Substance	Animal, Route	LD^{50}
Water	rat, oral	90 mL/kg (~90,000 mg/kg)
Sucrose (table sugar)	rat, oral	29,700 mg/kg
Vitamin C (ascorbic acid)	rat, oral	11,900 mg/kg
Grain alcohol (ethanol)	rat, oral	7,060 mg/kg
Table Salt	rat, oral	3,000 mg/kg
Paracetamol (acetaminophen)	rat, oral	1,944 mg/kg
THC (main psychoactive substance in Cannabis)	rat, oral	1,270 mg/kg males; 730 mg/kg females
Aspirin (acetylsalicylic acid)	rat, oral	200 mg/kg
Caffeine	rat, oral	192 mg/kg
Nicotine	rat, oral	50 mg/kg
Strychnine	rat, oral	16 mg/kg
Sodium cyanide	rat, oral	6.4 mg/kg
Aflatoxin B1 (from Aspergillus flavus)	rat, oral	0.048 mg/kg
Dioxin (TCDD)	rat, oral	0.020 mg/kg
Batrachotoxin (from poison dart frog)	human, sub-cutaneous injection	0.002-0.007 mg/kg (estimated)
Polonium 210	human, inhalation	0.00001 mg/kg (estimated)
Botulinum toxin (Botox)	human, oral, injection	0.000001 mg/kg (estimated)
Substance	Animal, Route	LC_{50}
Alkyl dimethyl benzalkonium chloride (ADBAC)	fish, immersion	280 µg/L