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Editorial

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Classifying chemicals into toxicity categories based on LC_{50} values- Pros and cons

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It is a usual practice to determine LC_{50} value in acute toxicity studies conducted in aquatic organisms as an initial step to assess the toxicity of chemicals. In regulatory toxicity studies, normally conducted in GLP (Good Laboratory Practice) certified facilities, acute toxicity of chemicals is evaluated in fish, crustacea, and or alga following the methods given in OECD Guidelines. The chemicals are classified into different toxicity categories based on the LC_{50}/EC_{50} determined from the acute toxicity studies. For calculating LC_{50} in acute toxicity tests, the methods given in the OECD (2019) Guidelines are Probit or Logit Analysis (Litchfield & Wilcoxon method and Probit Analysis), Spearman-Karber method, the binomial method, the moving average method, and the graphical method. LC_{50} is the concentration of a substance that causes 50 % mortality in a batch of test organisms (eg. fish). In acute toxicity studies with laboratory animals like rats, mice, rabbits, etc, instead of LC_{50}, the terminology LD_{50} is used. The procedure for the calculation of both LC_{50} and LD_{50} is same. In this article, LC_{50} and LD_{50} are written interchangeably. It means if 100 fish are exposed to LC_{50}, theoretically 50 fish would die. In fact, the inventor of LC_{50} (Trevan, 1927) defined LC_{50} as the median lethal concentration. Like any other median value, the LC_{50} is not affected by extreme values of either side. Unfortunately, Trevan was ruthlessly misquoted by the animal ethicists, as they believed that he was responsible for killing millions of animals for determining the median lethal concentration. According to Rowan (1983), the median lethal concentration in animals varies considerably among the species and is affected by environmental factors. Trevan proposed median lethal concentration (LD_{50}) in frogs and rodents for biological standardization of digitalis extract, insulin, and diphtheria toxin when he was working at Wellcome Research Labs, Beckenham (Pillai *et al.*, 2021a). Trevan never promoted sacrificing more animals to determine median lethal concentration. He was aware of the fact that the determination of median lethal concentration is affected by several factors. The 'characteristic' of a dose-response curve proposed by Trevan is species and test substance-specific. However, after Trevan, LD_{50}s were determined in acute toxicity studies to evaluate the effect of a substance, not for the biological standardization of drugs. His intention was to establish a numerical quality control standard to assess batch-to-batch variation, if any, of the therapeutic products of the Wellcome Research Labs.

Based on the LC_{50}/EC_{50} values determined in aquatic toxicity studies, the chemicals are classified into a hazard category. For example, according to United Nations Global Harmonized System (GHS), if the 96 LC_{50} of a chemical to fish is ≤ 1 mg l^{-1}, this chemical is classified into hazard category I (GHS, 2019).Though several methods are prescribed in OECD (2019) Guidelines, if the mortality data are adequate, Probit Analysis of Finney (1978) and Litchfield and Wilcoxon (1949) method may be preferred to determine LC_{50} as these methods provide additional valuable information on the concentration-mortality relationship. If the lowest mortality obtained is close to 16% and the highest mortality is close to 84%, most of the above-mentioned methods would result in a more or less similar LC_{50}value (Pillai *et al.*, 2021a).Calculation of LC_{50} manually by the Litchfield and Wilcoxon method is somewhat easier, but Probit Analysis is a bit cumbersome. Commercial statistical software is available for the calculation of LC_{50} by both the above methods. But, using the software without understanding the underlying concepts of the statistical methods has certain disadvantages. Researchers also present the toxicity of a substance in terms of LC_{10}, LC_{90}, etc. Since the variability of these estimates is large, their biological relevance is limited. Concentration-mortality curve in the 16-84% mortality range is linear, hence the LC_{50} determined from this concentration-mortality curve is reliable. The method of Litchfield and Wilcoxon (1949), uses the 16-84% mortality range for calculating LC_{50}. This method does not consider mortality below 16 and above 84% for the LC_{50} calculation. But Probit Analysis by Finney (1978) considers all mortality values (excluding 0 and 100 % mortality) for the calculation of LC_{50}.

Researchers in academic institutions use LC_{50} values to compare the toxicity of the test substances - the lower the LC_{50}, the substance is more toxic, and *vice-versa* (Islam *et al*., 2021).Toxicity grading of substances solely based on LC_{50} is inappropriate. Recently, the appropriate use of LC_{50} values for the GHS classification of chemicals has been questioned (Pillai *et al*., 2021a). LC_{50}s vary in a wide range from one species to the other (Geyer *et al*., 1993) and many times are irreproducible within the same species (Peres and Pihan, 1991), as the physico-chemical parameters of dilution water play a crucial role in LC_{50} experiments. Hrovat *et al.* (2009) reported significant variability of fish LC_{50} test results for 44 compounds. A consistent LC_{50} could not be obtained in more than 750 tests conducted on fathead minnows with 644 chemicals (Mc Carty, 2012).

It is a statutory requirement for the United Nations GHS that the environmental hazards should be mentioned on the labels of chemicals for distribution. The European Chemicals Agency (ECHA, 2017) uses fish LC_{50} for the environmental classification of a chemical according to the GHS of Classification, Labelling and Packaging of Chemicals (Paparella *et al*., 2021). The major disadvantage of such labelling is that the LC_{50} value alone does not provide information on the toxicity profile of chemicals. Showing a similar LC_{50} does not mean that the toxicity profile of the chemicals is same. It is important to consider the slopes of the concentration-mortality curve when comparing the LC_{50}s of the chemicals. The slope which reflects the concentration-mortality relationship provides a better understanding of the causality between a toxicant and response (Tsatsakis *et al.,* 2018). In Probit Analysis, parallel regression lines of mortality probits on log concentrations indicate that the mode of action of chemicals on test organisms is similar (Finney, 1978). If the regression lines are not parallel, it is a clear indication that the chemicals possess different modes of action on that particular organism. Also, it is important to present LC_{50} with 95% confidence limits. If the 95% confidence limits of LC_{50}s of the chemicals are distinctly separate, LC_{50}s can be considered different from each other. The LC_{50}s cannot be considered different from each other if the 95% confidence limits of the LC50s overlap. Chemicals with similar LC_{50} values may manifest toxicity differently. Similarly, chemicals with different LC_{50} values may manifest similar toxicity effects; hence, the classification of chemicals into various groups based on LC_{50} values may not have much relevance (Pillai *et al*., 2021b).

Ethical conduct of fish toxicity studies and euthanizing of exposed fish are emphasized in the OECD (2019) and CCSEA (Committee for Control and Supervision of Experiments on Animals) Guidelines (CPCSEA, 2021). Earlier the fish toxicity studies were conducted with 10 fish exposed to each test concentration, but the revised OECD (2019) Guideline recommends a minimum number of 7 fish for each test concentration. The probable mortality data that can be obtained in an acute test where 7 numbers of fish are exposed to each test concentration are (number of fish died/total number of fish exposed) 0/7, 1/7, 2/7, 3/7, 4/7, 5/7, 6/7, or 7/7. For calculating LC_{50} values by the methods of Litchfield and Wilcoxon (1949) and Finney (1978), 0 and 100% mortality are not used, since no probit values can be assigned for 0 and 100% mortality. The remaining 6 numbers of mortality data are adequate for calculating a reliable LC_{50} value, if the mortality data spreads over all phases of the concentration-mortality curve, particularly covering 16-84% mortality region. If the mortality data does not spread over all the phases of the concentration-mortality curve in a concentration-dependent manner, the confidence limits of LC_{50} could be exploded (Pillai *et al*., 2021b).

Estimation of LD_{50} in rodents by the methods of Litchfield and Wilcoxon (1949) and Finney (1978) is discouraged by US Consumer Product Safety Commission, US Environmental Protection Agency, US Food and Drug Administration, National Toxicology Program, and OECD, due to ethical reasons and poor reproducibility of LD_{50} values. But, classical methods are used to determine LC_{50} values in environmental toxicity studies, especially with aquatic organisms. It is more biologically relevant to interpret LC_{50} in terms of the slope of concentration-mortality curve and confidence interval of LC_{50}.

My association with Dr. R.C. Dalela and *Journal of Environmental Biology* began in the early 1980s when he was working at D.A.V College Muzaffarnagar. His research work and enthusiasm for bringing up the *Journal of Environmental Biology* to an international standard fascinated me. I realized from his research work that he was a committed environmentalist. I had an opportunity to majorly organize two national conferences of the Academy of Environmental Biology. He always occupied the front row in the conferences listening to all scientific presentations keenly. He had taken a lot of hardships to bring the journal to this sustainable level with a WOS Impact Factor of 0.70. I remember as it had happened yesterday, my meeting with him at D.A.V. College, Muzaffarnagar, at JRF, Vapi, Marathwada Ambedkar University, Aurangabad, and in Chennai. He was an excellent teacher, a great scientist, a mentor to several researchers, and self-disciplined.

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