

## **Pesticide Toxicity Indices for Aquatic Pollution Impact Assessment: Limitations and Need**

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Though in last few decades wealth of information on pesticide toxicity to fishes has been accumulated due to their increased application, it is intriguing that the definition and methods for toxicity assessment still lack precision and handiness. Majority of the studies concerning effects of pesticides on fish have been confined to the acute toxicity tests with death of fish as an endpoint. However, it is evident that the fish ceases to function normally long before this end point is reached. It has now been well established that pesticides even in traces interfere with various physiological and metabolic processes. Pesticidal hazards to fish range from impaired physiology, biochemistry, behaviour, growth, reproduction, etc., to mortality.

The prevailing terminologies to express toxicity of pesticides are maximum acceptable toxicant concentration (MATC), median tolerance limit (TLM), median lethal concentration (LC 50), initial lethal concentration [LC(I)50], sub lethal concentration (SL) and safe concentration (SC). According to Mount and Stephan (1967), MATC is the highest concentration of pesticide under chronic exposure condition that has no effect on reproduction, growth, spawning, behaviour, egg viability and fry survival. But it is hard to accept that MATC has no effect on the above physiological processes without exploring the events at molecular level. Mount and Stephan (1967) have also proposed laboratory fish production index (LFPI) as the most effective biological measure that can be used in laboratory for toxicity assessment. However, on many accounts obtaining permissible concentration of pesticide the suggested indices are not satisfactory.

There are two major problems which do not allow reaching any agreement on the permissible concentration of any pesticide for fish. The first one is the lack of application of laboratory data to field due to inadequate field studies, and the second greatest difficulty lies with the inabilities of subject experts to agree on the type of effect to be measured or pesticides invoked damages as an index. In fact, the most common way of directly assessing potential effects of pesticides is through the use of standard laboratory toxicity tests that expose a single species to a single pesticide over a range of concentration for a specified period of time. The comparison of such toxicity results among fishes indicates the relative toxicity of these pesticides under standard test conditions. However, it doesn't consider the factors that are important in extrapolating to

field condition. Such factors include the endpoint selected, environmental factors (like organic carbon, pH, temperature, photoperiod etc.), and potentials for additive or interactive effects of contaminant mixture in field.

Apart from information the toxicity limit of various pesticides to fish, voluminous reports on sub-lethal effects of pesticides also exhibit contradictions. Differences exist because of the lack of uniformity in the experimental design, condition and species used. Moreover, fishes encounter circadian and circannual fluctuations in the external environmental cues, and because of these fluctuations internal milieu of fish changes constantly (thus the interacting factors), which collectively determine the nature and extent of response to any pesticide.

Observations on fish mortality in response to various pesticides are very frequently reported using the conventional indices such as maximum acceptable toxicant concentration (MATC), median tolerance limit (Tlm) and median lethal concentration [LC(I)50]. Murty (1982, 1983) has reviewed toxicity limit of various pesticides extensively for a quite large number of teleost. There are basically two systems for assessing the toxicity of any pesticide; static test and the flow through test. The former constitutes a stationary system in which pesticides are present either in dissolved or suspended form, while in the latter, there is continuous flow of water with pesticide dissolved in it. The results of static test are debatable because the concentration of pesticide does not remain constant throughout the duration of experiment (Holden, 1962; Lincer *et al.*, 1970). Hence, flow through system has advantages over static one. However, facilities for flow through assays are not available in most of the laboratories in developing countries; therefore, static bioassay tests are still prevalent. The conventional terminologies are inadequate indices for acute toxicity limits because the catastrophe of death obscures not only the mechanism and site of action. But they also do not include the evaluation of nonlethal effects. These indices represent only short-term effect in terms of death. They do not measure the long-term effects including impairment in physiologies, embryonic development, teratogenic impacts. The prevailing indices are of marginal use and toxicologically inadequate and misleading too. But unfortunately in developing countries, these indices are very frequently used for the evaluation of toxicity and for calculating acceptable and permissible limits of pesticides for their registration in different government agencies and pollution boards. Persons concerned with abatement of fish pollution often encounter authorities who are not willing to agree with the thesis that the environment is detrimental to fishes just because pesticides produce adverse impacts on normal functioning.

Pollution biologists must have enough quantitative data to prove that the observed changes resulting from exposure is ecologically detrimental to fishes. An exposure causing death is obviously significant, but even the best fish physiologist would have difficulty in establishing that 10% reduction in hematocrit value would result in undesirable effects on a population. Even a reduction in growth during 30

days exposure might be explained as a transit effect that would be insignificant over a long period. Mount and Stephan (1967) have proposed laboratory fish production index (LFPI) as the most effective biological measure that are used in laboratory for toxicity assessment. According to them under, chronic exposure condition, the highest pesticide concentration that has no effect on reproduction, growth, spawning behaviour, egg viability and fry survival is termed as MATC. They have further suggested that MATC for a species can be used to calculate application factor for other species also, which cannot be tested in laboratory. Application factor is calculated by dividing MATC by 96 h TLm. The term TLm is also designated as TL 50 (median tolerance limit) (Carlson, 1971; McKim and Benoit, 1971) or LC 50. (Toor and Kaur 1974; Mayer *et al.*, 1975). Since experimental protocol of these authors were not in full agreement with Mount and Stephan (1967) for evaluation of MATC and TLm. They used different terminology such as safe concentration (SC) - the highest concentration of pesticides under which fish does not exhibit any sign of stress; sub-lethal concentration (SL) - the highest pesticide concentration at which there is no mortality though fishes are seen in stress; and the initial median lethal concentration [LC(I)50] or lethal concentration (LC50) or effective concentrations (EC50) - the concentration of pesticide causing 50% mortality. Battaglin and Fairchild (2002), Guy *et al.* (2011), Munn and Gilliom (2001), Munn *et al.* (2006), Schäfer *et al.* (2011a, 2011b), Stenström (2013) also used these indices. The toxicity unit are also calculated as chronic no observed - effect concentrations (NOECs) or LC50/EC50s multiplied by a safety factor to represent chronic effects (Anderson, 2008), water-quality standards (Stenström, 2013), and hazardous concentrations (HC) derived from species sensitivity distributions (SSD) (Guy *et al.*, 2011; Maltby *et al.*, 2005, 2009; Sala *et al.*, 2012; Schäfer *et al.*, 2013; Whiteside *et al.*, 2008). A SSD entails fitting a statistical distribution to toxicity data for certain broad taxonomic groups, and SSDs are constructed from acute LC50/EC50 values (Schäfer *et al.*, 2013; Whiteside *et al.*, 2008), chronic NOEC values, or any other selected toxicity criterion (Posthuma and de Zwart, 2006). Recently, Nowell *et al.* (2014) have proposed another index for pesticide impact assessment index, Pesticide Toxicity Index (PTI) as robust and readily available screening tool for the interpretation of the biological significance of concentration data for pesticide mixture in hydrologic systems. The PTI system too has many limitations as it is a relative system based on short-term laboratory experiment with non-lethal response or mortality endpoint. It does not reflect long-term/ chronic exposure. It also does not account for environmental factors like dissolved organic carbon, particulates, pH, temperature, photoperiod, which affect the toxicity and bioavailability of pesticides.

Moreover using these indices enough information on toxicity limit of various pesticides has been assayed but results are highly ambiguous owing to various contradictions. Differences exist due to the lack of the uniformity in experimental design and species used. Studies have been performed either *in*

*vivo* or *in vitro* condition. Some workers have chosen one criterion under a particular set of conditions, while others have selected different criteria under different set of conditions leading obviously to varied results. Differences *in vivo* and *in vitro* effects of pesticides, which are frequently seen in literature pose another problem. Considering inconsistencies between *in vivo* and *in vitro* responses, Bostrom and Johansson (1972), and Scultz and Ardman (1989) have suggested that *in vitro* responses cannot necessarily reveal the true pesticides induced stress under natural condition.

Physiology of living organism is the manifestation of intricate and complex biochemical processes. Fish encounter circadian and circannual fluctuations in the external macro-environmental cues e.g. photoperiod, temperature, hydrophysico-chemical factors etc. which are perceived by fish through humoral/neuro hormonal/hormonal messages. These messages at various target tissues are translated into different biochemical reactions regulating physiology of the tissue. Because of variation in environmental factors, the internal milieu of fish changes constantly and thus all the above interacting factors altogether dictate the nature and extent of the response of fish to any pesticide. The basis of any activity including response of fish to any pesticide lies in the biochemical action and reaction inside the body. Most toxicants exert their effect at the molecular level of the organism by reacting with enzymes and metabolites in enzymatic reactions, or by binding to and interacting with membrane structure or other functional components of the cell. Such primary interactions between the toxic substance and various cell components may induce a sequence of structural and functional alterations at a higher level of organization, manifested by impairment of vital functions, such as nerve and muscle function, respiration, circulation, immunity, defense, osmoregulation and hormonal balance. Thus, it would be advantageous to emphasize on pesticide-induced alterations in biochemical and molecular profiles of fishes instead of restricting to toxicity limits taking death as endpoint. This approach will converge the scattered information into principles. Altered biochemical profiles following pesticide exposures usually lead to irreversible and detrimental disturbances of integrated functions such as behaviour, digestion, growth, reproduction, and above all survival, which in turn may lead to the changes at population level.

Thus, considering the above facts and serious limitations in prevailing indices used for pesticide impact assessment for fishes, an immediate attention is warranted to define and evolve method for environmental impact assessment which should be scientifically sound to the workers in the field.

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