Aquatic Toxicity Testing Of Difficult Substances by Water Accommodated Fraction (WAF)

Conducting aquatic eco-toxicological studies on difficult substances is quite a challenging task. Specifically, sample preparation in test media needs focus and use of inappropriate method could result in an ambiguous end points. The selection of right approach, after due consideration of the property of difficult substances, gives accurate result. The approach through which a test solution is prepared (based on the properties of the test substance) and exposed to the test system, as well as the treatment of any undissolved test substance, can have a significant impact on results and reliability of the study. A few properties of difficult substances include its poor water-solubility, toxicity at low concentration levels, volatility, photo-degradability, hydrolytical unstability, oxidisability, biodegradability, adsorbing ability, complexing nature, colour,hydrophobicity, ionisation, and multi-components substances and its preparations. An approach to handle mixture of sparingly soluble components and their preparations for aquatic studies is focused on, in this newsletter.

Water accommodated fraction approach is applied to the aqueous media, containing only a fraction of multi-component substances, dissolved and/or present as a stable dispersion or emulsion.

There are a number of parameters involved in sample preparation using WAF approach. Optimisation of these parameters with respect to the nature of sample is a critical step and is carried out in the JRF with an utmost care. During the process of WAF preparation, the duration of mixing and re-equilibrium time is determined by carrying out a preliminary study. Water-miscible solvents are not used during preparation, as they may modify the composition of the WAF. It is done by standardisation of mixing and re-equilibrium time by anlysing active ingredient at different intervals, e.g., 1, 3, 6, 24 h. Prior to adding the volume of test solution used for exposure to test vessels, these are pre-conditioned (rinsed) with respective test concentration levels to saturate the surface of the respective vessel to prevent loss of test concentration due to absorption to walls of test vessels. The test item is mixed with the test medium and kept for stirring at room temperature. After stirring, the test solution is kept for re-equilibrium. After phase separation, test solution is collected from the lower portion without disturbing the phase. It is important to recognise that the duration of mixing can have a marked influence on the composition, particle size, and proportion of the dispersed and non-dispersed test material in the WAF. Generally, non-dissolved test materials in the test vessel are removed. It is ensured that only a fraction of total mass of the multi-component substances, which may be responsible for the composition of a WAF, remain present in the WAF. Chemical specific analyses are required to demonstrate attainment of equilibrium in the WAF preparation and stability during the test. Measured amounts of multi-component substances are added directly to water and mixed for a period of time, sufficient enough to achieve an equilibrated concentration of dissolved and dispersed or emulsified components in the aqueous phase. Post cessation of mixing and a period of settling (allowing phase separation) the aqueous phase, i.e., the WAF, is drawn off for testing. Test data obtained with WAFs apply to the multi-component substances, as an entity.

Effect concentration levels, in tests based upon WAFs, can be reported as nominal concentration level either from loading rates and are identified as either LL_{50} or EL_{50} values and/ or the measured mass of test substance in the WAF and are identified as either LC_{50} or EC_{50} values. LL_{50} or EL_{50} values are comparable to LC_{50} or EC_{50} values determined for pure substance tested within its solubility range. Similarly the NOEC (No Observable Effect Concentration) becomes the NOELR (No Observable Effect Loading Rate). The statistical methods used to determine LL_{50}, EI_{50} and NOELR values are the same as those used to determine LC_{50}, EC_{50} and NOEC values.

Reference :

OECD, 2000: OECD Series on Testing and Assessment Number 23, OECD Principles on Good Laboratory Practice ENV/JM/MONO (2000)6.

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