Aquatic toxicity testing for petroleum substances

The EU Dangerous Substances Directive (DSD) provides a systematic approach for the classification of substances based on knowledge of their intrinsic properties and in accordance with defined hazard criteria. The 18th Adaptation to Technical Progress (ATP) of the DSD provides the specific criteria for the classification of substances for environmental hazards.

CONCAWE has based its recommendations for the environmental classification of petroleum substances on results from Water Accommodated Fraction (WAF) studies. Some regulatory bodies (GESAMP[1], UK Department of the Environment), along with industry associations (CEFIC, CONCAWE) and others, recommend the WAF approach as the most suitable method for testing the aquatic toxicity of 'difficult' substances. Its wider acceptance by EU Member States remains, however, under discussion.

Current OECD guidelines for the acute aquatic toxicity testing of substances are unsatisfactory when the substances contain components which are poorly soluble in water or volatile. However, during 1998, the OECD set up a working group to specifically address the problems of testing these 'difficult' substances with the aim of improving the current advice given in its ecotoxicity testing guidelines. The WAF method is included in a January 2000 OECD draft guidance document on the aquatic toxicity testing of difficult substances.

Test methods are only part of the problem of testing these difficult substances. Expression of the results is also problematic. All these difficulties are experienced in dealing with the aquatic toxicity testing of petroleum substances. Some of the problems encountered, and the solutions that have been found, are dealt with in the following paragraphs.

Because of the low water solubility of the hydrocarbon components, adding petroleum substances to water to produce solutions for the evaluation of aquatic toxicity rapidly results in the production of two-phase systems. A number of approaches have been developed to produce 'media' for testing, and these have previously been reviewed[2,3]. Many early studies described the preparation of water extracts of petroleum substances at very high loading rates (the term 'loading' or 'loading rate' has been frequently used as shorthand for the amount of hydrocarbon added to a specific volume of the test medium). The water extract was then diluted for testing, and the results expressed in terms of the percent dilution of the extract, or alternatively, in terms of the concentration of particular constituent hydrocarbons measured in the water. Data from this type of study, usually referred to as the WSF (Water Soluble Fraction) approach are not useful for classification; the percent dilution result is not in line with the quantitative criteria defined (expressed in terms of mg/l of test substance). Results based upon concentrations of specific dissolved components are again not suitable because the qualitative composition of the water fraction produced at a high loading rate may differ considerably from that of a solution prepared at a lower loading rate.

[1] *IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution*

[2] *Connell, D.W. and Hawker, D.W. (1988). Use of polynomial expressions to describe the bioconcentration of hydrophobic chemicals by fish.* Ecotox Environ Safety *16, 242–257.*

[3] *Girling, A.E. (1989). Preparation of aqueous media for aquatic toxicity testing of oils and oil-based products: a review of the published literature.* Chemosphere *19, 10–11, 1635–1641.*

Test methods based upon varying the loading concentration of the test substance in order to determine the toxicity, produce data more applicable to classification. Often, with petroleum substances composed primarily of low molecular weight hydrocarbons such as naphthas, nearly all of the substance is dissolved within the loading range where acute toxicity is observed. However, heavier petroleum substances contain a major proportion of components that are not completely soluble. In these cases, there are three methodological options available:

1. Remove the insoluble hydrocarbon portion;
2. Stir the mixture continuously to keep the insoluble material dispersed;
3. Allow the two phases to remain without physical mixing.

Approaches 1 and 2 have been most commonly utilized. The disadvantage of the second and third approaches is that the undissolved hydrocarbon may result in physical fouling and/or entrapment of the organisms, particularly *daphnia* and algae; the stirring apparatus used in the second approach may also cause physical harm to such small organisms. The first approach is favoured by CONCAWE, as it precludes physical effects and measures chemical toxicity. The water phase used in this approach is usually known as the Water Accommodated Fraction (WAF), since it is not usually possible to demonstrate that it is a true solution. Toxicity data resulting from these tests are expressed in terms of 'lethal loading' (LL) to distinguish them from the conventional lethal concentration (LC) data developed for single substances. The results of lethal loading studies are usually expressed as LL_{50} (the loading rate killing 50 per cent of organisms) or EL_{50} (the loading rate causing 50 per cent effect), rather than the corresponding LC_{50}, or EC_{50} values.

The concentration of each individual chemical species dissolved in the water phase at any particular 'loading' must be maximized. The maximum possible water concentration of each component is achieved through prolonged stirring of the water-petroleum substance mixture. In addition, a sealed system approach is necessary to measure the inherent toxicity of the more volatile petroleum substances.

Chemical analyses of the aqueous concentrations of all constituents are not possible due to the complexity of the composition, but equilibration should be confirmed by analysis of selected (representative) hydrocarbons or total dissolved hydrocarbons. Analyses are also typically undertaken to confirm whether the concentrations of selected components (and by inference, all components) decrease due to volatilization, or other processes such as biodegradation, during the period of exposure. Renewal of the WAF may be done daily, or at less frequent intervals, depending on stability. The concentrations of individual hydrocarbons in test media are not needed to calculate the LL_{50} value, since the results are expressed in terms of the entire substance (the concentration, or loading rate, used to make the initial WAF).

As a general rule, the use of auxiliary solvents or surfactants in the preparation of media for the testing of petroleum substances is not recommended. The presence of solvents during the preparation of test media from complex substances poses particular problems, as a consequence of their potential to influence partitioning between the dissolved and undissolved phases.

In conclusion, determination of the toxicity of petroleum substances is not a trivial matter. CONCAWE reaffirms its view based on sound technical arguments for the WAF method as the most suitable method for the aquatic toxicity testing of petroleum substances and strongly supports its regulatory acceptance by the EU.