

## Review on the acute



Executive summary : One of the most internationally used bioassays for toxicity screening of chemicals and for toxicity monitoring of effluents and contaminated waters is the acute toxicity test with daphnid crustaceans, and in particular that performed with *Daphnia magna*. Standard methods have been developed for this assay that were gradually endorsed by national and international organisations dealing with toxicity testing procedures, in view of its application within a regulatory framework. As for all toxicity tests, the organisms used for the acute *D. magna* assay have to be obtained from live stocks which are cultured in the laboratory on live food (micro-algae). Unsurprisingly the various standard protocols of this particular assay differ – at least to a certain extent – with regard to the test organism culturing conditions. In addition, some technical aspects of the toxicity test such as the effect criterion (mortality or immobility), the exposure time, the type of dilution water, etc., also vary from one standard to another. Although this particular assay is currently used in many countries, the technical and biological problems inherent in year-round culturing and

availability of the biological material and the culturing/maintenance costs of live stocks restrict its application to a limited number of highly specialised laboratories. This fundamental bottleneck in toxicity testing triggered investigations which brought forward the concept of “microbiotests” or “small-scale” toxicity tests. “Culture/maintenance free” aquatic microbiotests with species of different phylogenetic groups were developed in the early 1990s at the Laboratory for Environmental Toxicology and Aquatic Ecology at the Ghent University in Belgium. These assays which were given the generic name “Toxkits”, are unique in that they employ dormant stages (“cryptobiotic eggs”) of the test species, which can be stored for long periods of time and “hatched” at the time of performance of the assays. One of these microbiotests is the Daphtoxkit F magna, which is currently used in many laboratories worldwide for research as well as for toxicity monitoring purposes. The microbiotest technology has several advantages in comparison to the “traditional” tests based on laboratory cultures, especially its independence of the stock culturing burden. However, the acceptance (or possible non-acceptance) of performing assays with test organisms obtained from “dormant eggs” should be clearly dictated by the “sensitivity” and “precision” criteria of the former assays in comparison to the latter. The first part of this review therefore thoroughly reviews the scientific literature and of data obtained from various laboratories for assays performed with either *D. magna* test organisms obtained from lab cultures or hatched from dormant eggs. Attention has focused on data of quality control tests performed on reference chemicals, and in particular on potassium dichromate ( $K_2Cr_2O_7$ ) for which an acceptability range of 0.6–2.1 mg·L<sup>-1</sup> has been set in ISO standard 6341 for the 24 h EC50 of the acute *D. magna* assay. Mean EC50s, standard deviations and variation coefficients were calculated from the collected data, all of which are presented in tables and figures and discussed in detail. The major conclusions drawn from the analysis of the large number of quality control (QC) data on the acute *D. magna* toxicity test are that : (1) Virtually all results from assays performed with *Daphnias* taken from lab cultures or with *Daphnia* microbiotests are within the acceptability range set by ISO standard 6341 for the reference chemical potassium dichromate. (2) The mean 24 h EC50s of the *Daphnia* microbiotests performed in different laboratories are within the range of the mean EC50s of the assays based on lab cultures, and the variation coefficients (20 to 30%) are similar. (3) The precision – in terms of the long term in house variability – of the quality control *Daphnia* microbiotests is as good as that of the QC tests based on lab cultures. The review further reports on intra-laboratory sensitivity comparison studies performed during the last 15 years on pure chemicals and on natural samples, with both laboratory cultured organisms and *Daphnias* hatched from dormant eggs. These studies carried out in different laboratories showed EC50 correlation coefficients of 0.86 to 0.98, corroborating a similar sensitivity of the two types of test organisms. The third part of the review reports and analyses data on proficiency ringtests on the acute *D. magna* assay which have been organised in different countries since 2002 with either reference chemicals or with natural samples, and in which part of the laboratories performed their assays with *Daphnia* microbiotests and others with lab cultured *Daphnias*. The conclusions drawn from all the ringtests indicate that the sensitivity of *Daphnia* neonates hatched from dormant eggs is similar to that of test organisms taken from lab cultures and that in most cases the precision of the *Daphnia* microbiotest is superior to that of the assays based on lab cultures. The review finally addresses the issue of possible sensitivity differences of *Daphnias* hatched from dormant eggs which are produced by different *D. magna* strains. From these investigations it appeared that the EC50s from assays performed with *Daphnias* hatched from dormant eggs of different strains did not differ significantly from those from assays undertaken with daphnids from lab cultures. The obvious advantages of *Daphnia* microbiotests over tests with *Daphnias* stemming from lab cultures have led to the worldwide use of these culture/maintenance free and low cost small-scale assays in both research and toxicity monitoring. The *Daphnia* microbiotest is in current use in several countries for toxicity testing in a regulatory framework, and recent calculations indicate that about 10 000 acute *D. magna* assays are now performed annually with neonates hatched from dormant eggs. The use of dormant eggs to obtain test organisms independently of stock culturing has recently also been accepted in international standards for toxicity testing. ISO standard 20665 (2008) related to the determination of chronic toxicity with *Ceriodaphnia dubia*, and ISO standard 20666 (2008) for the determination of the chronic toxicity with *Brachionus calyciflorus* in 48 h, both indicate that the assays can be conducted with organisms hatched from dormant eggs. On the basis of the extensive scientific evidence provided in this review that is justifiably supported by the two ISO methods mentioned above, the authors therefore recommend that the use of *Daphnias* hatched from dormant eggs should also be incorporated in national and international standards, as an alternative to the use of *Daphnias* taken from laboratory cultures.

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