Environmental Research Applications

In virtually every country around the world there is concern about the increasing number of new chemicals that are entering our environment. Many of these chemicals have been tested for acute toxicity prior to their being approved for use in society. Many of these chemicals, however, undergo changes as they move through societal use and through the natural environment when they are:

- Recycled in water systems
- Incinerated
- Decomposed in landfills
- And are modified by microorganisms in surface waters, ground waters, soils or sediments

In a number of universities and research institutes, scientists are screening for the presence of acutely toxic, genotoxic or mutagenic materials so that they can be isolated, identified and environmental and public health risks evaluated. EBPI test kits are being used in over 30 countries in these applications around the world.

Toxi ChromoTest™ Kit - Testing For Toxicity

This test kit is simple and easy to run in a standard laboratory or simple field laboratory. It can be used to test for the presence of toxic materials in water and a special kit has been developed for the direct testing of soils, sludge's or sediments for the presence of toxic materials. The kit measures the ability of a rough mutant of E.coli to activate the LacZ gene and excrete beta galactosidase into solution where its presence is measured by its reaction with a chromogen. The test thus has a simple colourimetric endpoint. If toxicity is detected further sample fractionation ,using standard TIE (Toxicity Identification Evaluation) protocols, can be undertaken to isolate and identify the responsible material(s). Good laboratory practice should be followed as with all testing using bacteria.

SOS ChromoTest™ Kit - Testing For Genotoxicity

This test has been designed so that the bacteria is grown the evening prior in which the test is to be carried out and the test can easily be completed within a three hour time period the following day. The test relies on the use of a genetically engineered E. coli bacterium in which the promoter for the SOS gene repair complex has been linked to the LacZ gene. The theory is based upon the fact that in all cells when DNA is damaged by a “genotoxic agent” the cell tries to repair the damage through the induction of one or more DNA repair systems such as the SOS system. In this test when the DNA of the test bacteria is damaged the bacteria “tries to activate” the SOS gene repair complex, but because the promoter that turns on the SOS system has been linked to the LacZ gene, the Lac Z gene is activated instead and the enzyme beta galactosidase is produced and excreted into the growth well. A chromogen is introduced to the growth well and if the cell has suffered DNA damage and it is “trying to repair it” using the SOS repair system a blue color will be produced. The test kit is supplied with a positive and negative control to ensure that acute toxicity has not occurred during the test and that it is functioning properly. The strength of the response of “unknowns” can be directly compared with the response of the “known” genotoxic positive control. The appearance of

genotoxicity following metabolism can also be determined through the activation of genotoxicity following exposure to the S-9 liver enzyme. The laboratory requires only a micro pipette, a 37 0 C incubator, bench space, a spectrophotometer to measure the bacteria density in the test suspension and an autoclave or chlorine bath to kill the bacteria following the testing. Good laboratory practice should be followed as with all testing using bacteria.

Muta Chromoplate™ Test - Kit Tests for Mutagenicity Based on the 'Ames Test'

This test kit has been designed to determine if material being tested results in a significant increase in mutation rate above background rates. The test will be set up on the first day and the results read after the bacteria have been allowed to grow and mutate for a five day period. The test is based upon the AMES “reverse mutation” assay where a mutation in the wild strain of Salmonella has resulted in the loss of the bacteria’s ability to metabolize

histidine. As the bacteria “reverse mutate” back, they gain the ability to metabolize the histidine present in the growth media and this results in a color change in the wells in the microtiter plate in which the bacteria are growing. A simple chart is provided by which a statistically significant difference between the natural background rate of mutation and the rate of mutation when exposed to different chemicals can be determined. The TA-98 and the TA-100 strains are the most commonly used but other strains are available. The test can also be used with S-9 activation if the formation of mutagenic materials through the metabolism of less mutagenic materials is suspected. The test kit comes with a positive control compound.

The laboratory requires only a micro pipette, a 37˚C incubator, bench space, and an autoclave or chlorine bath to kill the bacteria following the testing. Good laboratory practice should be

followed as with all testing using bacteria. Please contact EBPI for university protocols and laboratory designs.