Drosophila Mutagenicity Test

A combined assay of the DNA repair test and the wing spot test in *Drosophila melanogaster*

Introduction

The *Drosophila* mutagenicity test, which was developed by Inoue in Mitsubishi Chemical Corporation, is a combined assay of two mutaganicity testings using *Drosophila melanogaster*; the DNA repair test and the wing spot test (somatic mutation and recombination test). The DNA repair test can detect a DNA-damaging activity by assessing killing sensitivity of DNA repair-defective flies compared to DNA repair-proficient counterparts. The wing spot test can detect "loss of heterozygosity" caused by a somatic mutation and/or chromosomal recombination/deletion, by examining the incidence of mutant wing spots.

It is well known that mutagenic compounds are converted to reactive compounds by metabolizing enzymes such as cytochrome P-450s and that *Drosophila* also has metabolizing enzymes involved in metabolic activation of pro-mutagens. In the combined assay, the Oregon R(R) strain carrying a high capacity of bioactivation is utilized to increase the test sensitivity to genotoxic compounds requiring metabolic activation.

The experimental procedures described below are presented as an example of genotoxicity screening for chemical compounds in *Drosophila*.

Experimental Procedure (Example)

(1) Test and control substances

- Test substance
- Negative control (vehicle): aqueous solution containing 2% ethanol and 1% Tween 80
- Positive control: urethane

(2) Tester strains

- Strain A: *mei-9*^{a} *mei-41*^{D5} / FM7 ^{c}; *mwh*

  The X chromosomal genes, *mei-9*^{a} and *mei-41*^{D5}, are identified as excision-repair defective and post-replication repair defective mutations, respectively. The *mwh* gene on the third chromosome is a wing cuticle marker which confers multiple wing hairs in a single cell.

- Strain B: Oregon R(R)

  Wild type for the DNA repair capability and the wing hair morphology with a high bioactivation

(3) Preparation of treatment medium

The test substance will be dissolved or suspended in the vehicle at the appropriate highest concentration and then diluted with the vehicle. Urethane will be dissolved in the same vehicle at 1 mg/mL. The treatment media for larvae will be made by mixing the *Drosophila* instant media (1.2 g) with 4 mL each of test substance preparation. The instant medium mixed with the vehicle alone or urethane solution is served as negative or positive controls, respectively.

(4) Test method

Virgin females of strain A will be mated with males of strain B and allowed to lay eggs for 8 hours in the glucose medium. Three days after egg-laying, the F1 larvae will be harvested with a 20% glucose solution. The larvae floated on the surface of glucose solution will be harvested and washed with distilled water. Equal batch of the larvae will be introduced into the vials to allow feeding on the treatment media. Surviving F1 flies will be subjected to the following examinations.

<DNA repair test>

The recovered F1 flies will be classified into the following three classes of phenotypes and scored: DNA repair-proficient females with red round or bar eyes (F), DNA repair-defective males with yellow round eyes (M1), and DNA repair-proficient males with whitish bar eyes (M2). The survival of each class of flies is expressed as a relative number of flies from treated culture to that from negative control culture. The test substance is judged as positive for DNA-damaging activity when the survival ratio, M1/F and M1/M2, is less than 0.1 and 1, respectively, at any one concentration.

<Wing spot test>

The wing specimens will be prepared from F1 *mwh*/+ females with red bar eyes (heterozygotes for *mwh* gene). The wings will be inspected under a microscope for the mutant *mwh* spots. The numbers of wings examined are 100 wings in the negative control group, 50 wings for each concentration in the test article group, and 10 wings in the positive control group. The *mwh* mutant spots are classified into “small spot” consisting of one or two mutant cells and “large spot” consisting of 3 or more mutant cells. The spot frequency is expressed as a number of *mwh* spots per wing. The test substance is judged as positive when a significant increase of the large spot frequency is noted with a statistical method of Kastenbaum and Bowman[3)].

(5) Acceptance criteria

1) Positive control shows a clear increase in killing sensitivity in the DNA repair test, and a significant increase in the number of wing spot.
2) Negative control shows no increase in killing sensitivity in the DNA repair test, and no increase in the number of wing spot even when compared to the historical control values.

(6) Judgment criteria

The test substance will be judged as mutagenic in *Drosophila*, when it produces a positive result in the DNA repair test and/or the wing spot test.

3. References

1) Fujikawa, K (1993): Genotoxic potency in *Drosophila melanogaster* of selected aromatic amines and polycyclic aromatic hydrocarbons as assayed in the DNA repair test, Mutation Research, 290, 175-182.
2) Graf, U., F.E. Wurgler, A.J. Katz, H. Frei, H. Juon, C.B. Hall and P.G. Kale (1984): Somatic mutation and recombination test in *Drosophila melanogaster,* Environmental Mutagenesis, 6, 153-188.
3) Kastenbaum, M.A. and K.O. Bowman (1970): Tables for determining the statistical significance of mutation frequencies, Mutation Research, 9, 527-549.