

**"Genetic Toxicology: Mouse Spot Test"****1. INTRODUCTORY INFORMATION**• Prerequisites

- Solid, liquid, vapour or gaseous test substance
- Chemical identification of test substance
- Purity (impurities) of test substance
- Solubility characteristics
- Melting point/boiling point
- pH (where appropriate)
- Vapour pressure data (if available)

• Standard documents

There are no relevant international standards.

2. METHOD**A. INTRODUCTION**

The mouse spot test detects presumed somatic mutations in fetal cells following transplacental absorption of the test substance.

• Principle of the method

This is an *in vivo* test in mice in which developing embryos are exposed to a chemical. The target cells in the developing embryos are melanoblasts, and the target genes are those which control the pigmentation of the coat hairs. The developing embryos are heterozygous for a number of these coat colour genes. A mutation in, or loss of (by a variety of genetic events), the dominant allele of such a gene in a melanoblast results in the expression of the recessive phenotype in its descendant cells, constituting a spot of changed colour in the coat of the resulting mouse. The frequency of such spots in treated groups is compared with their frequency in the control group.

B. DESCRIPTION OF THE TEST PROCEDURE• Preparations***Test substance***

When possible, test substances are dissolved or suspended in isotonic saline. Chemicals

insoluble in water are dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be used.

Experimental animals

Mice of the T strain (nonagouti, a/a; chinchilla, pink eye, c^{ch}/c^{ch} ; brown, b/b; dilute, short ear, d se/d se; piebald spotting, s/s) are mated either with HT strain (pallid, nonagouti, brachypody, pa a bp/pa a bp; leaden fuzzy, ln fz/ln fz; pearl pe/pe) or with C57/B1 (nonagouti, a/a). Other appropriate crosses such as between NMRI (nonagouti, a/a; albino, c/c) and DBA (nonagouti, a/a; brown, b/b; dilute d/d) may be used provided they produce nonagouti mice.

Number and sex

Sufficient pregnant females are treated to provide an appropriate number of surviving mice at each dose level used. The appropriate sample size is governed by the number of spots observed in the treated mice and the scale of the control data.

Test conditions

Route of administration

The usual routes of administration are oral intubation or intraperitoneal injection of the pregnant females. Treatment by inhalation or other routes of administration are used when appropriate. The intraperitoneal route may be appropriate for an assessment of inherent mutagenicity. Maximum utility for risk assessment is obtained when the route of administration is relevant to human exposure.

Dose levels

At least two appropriately spaced dose levels are used including one producing signs of toxicity or reduced litter size. A relatively non-toxic substance should be tested up to 1 g/kg, or, if this is not practicable, up to the highest dose attainable.

Controls

Concurrent control data from mice treated with the vehicle only (negative control) should be available. Additionally, historical control data from the same laboratory, provided they are

"Genetic Toxicology: Mouse Spot Test"

homogeneous, may be used. When acceptable positive control results are available from experiments conducted recently (within the last twelve months) in the same laboratory, these results can be used instead of a concurrent positive control.

• Performance of the test

A single treatment is normally given on day 8, 9 or 10 of pregnancy, counting as day 1 the day on which the vaginal plug is first observed. These days correspond to 7.25, 8.25 and 9.25 days after conception. Successive treatments over these days may be used.

Analysis

The mice are coded and scored for spots between three and four weeks after birth. Three classes of spots are distinguished:

- a) white spots within 5 mm of the mid-ventral line which are presumed to result from cell killing (WMVS):
- b) yellow, agouti-like, spots associated with mammae, genitalia, throat, axillary and inguinal areas and mid-forehead, which are presumed to result from misdifferentiation (MDS); and
- c) pigmented and white spots randomly distributed on the coat which are presumed to result from somatic mutations (RS).

All three classes are scored but only the last, RS, is of genetic relevance. Problems of distinguishing between MDS and RS may be resolved by fluorescence microscopy of sample hairs.

3. DATA AND REPORTING

• Treatment of results

Data should be presented in tabular form.

The data are presented as the total number of mice scored and the number of spots presumed to be produced by somatic mutation. Data should also be presented on a per litter basis. The data are evaluated using appropriate statistical methods.

• Evaluation of results

There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the frequency of genetically relevant spots. Another criterion

may be based upon detection of a statistically significant positive response for at least one of the test points.

A test substance producing neither a statistically significant dose-related increase in the frequency of genetically relevant spots nor a statistically significant positive response at any of the test points is considered non-mutagenic in this system.

- Test report

The test report should include the following information:

- the strains used in the cross;
- the number of pregnant females in the experimental and control groups;
- the average litter size in the experimental and control groups at birth and at weaning;
- the dose level(s) of the test chemical;
- the solvent used;
- the day of pregnancy on which treatment was given;
- the route of treatment;
- the total number of mice scored, and the number of WMVS, MDS and RS in the experimental and control groups;
- the total number of litters scored, and the number with WMVS, MDS and RS in the experimental and control groups;
- gross morphological abnormalities, if observed;
- dose-response relationship of RS, if applicable;
- statistical evaluation;
- discussion of results;
- interpretation of results.

4. LITERATURE

R. Braun, L.B. Russell, and J. Schoeneich, Meeting report: Workshop on the practical applications of the mammalian spot test in routine mutagenicity testing of drugs and other chemicals, Gatersleben, GDR, *Mutation Res.* 97, 155-161 (1982).

L.B. Russell, The mouse spot test as a predictor of heritable genetic damage and other endpoints, in *Chemical Mutagens, Principles and methods for their detection* (edited by F.J. de Serres and A. Hollaender), pp. 95-109, Plenum Press, New York (1982).

L.B. Russell, P.B. Selby, E. von Halle, W. Sheridan and L. Valcovic, Use of the mouse spot test in chemical mutagenesis: Interpretation of past data and recommendations for future work, *Mutation Res.* 86, 355-379 (1981).