B. 24. MOUSE SPOT TEST

1. METHOD

1.1. Introduction

See General Introduction Part E.

1.2. Definition

See General Introduction Part E.

1.3. Reference substances

None.

1.4. Principle of the test method

This is an *in vivo* test in mice in which developing embryos are exposed to the chemicals. 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 number of offspring with these spots, mutations, are scored and their frequency is compared with that among offspring resulting from embryos treated with the solvent only. The mouse spot test detects presumed somatic mutations in foetal cells.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

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, $c^{ch}p/c^{ch}p$; brown, b/b; dilute, short ear, d se/d se; piebald spotting, s/s) are mated either with the HT strain (pallid, nonagouti, brachypody, pa a bp/pa a bp; leaden fuzzy, ln fz/ln fz; pearl pe/pe) or with C57BL (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 offspring.

Number and sex

Sufficient pregnant females are treated to provide an appropriate number of surviving offspring for each dose level used. The appropriate sample size in governed by the number of spots observed in the treated mice and the scale of the control data. A negative result is acceptable only when at least 300 offspring from females treated with the highest dose have been scored.

Use of negative and positive controls

Concurrent control data from mice treated with the vehicle only (negative controls) should be available. Historical control data from the same laboratory may be pooled to increase the sensitivity of the test provided they are homogeneous. Positive control data recently obtained in the same laboratory from treatment with a chemical known to show mutagenicity by this test should be available if no mutagenicity of the test chemical is detected.

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.

Dose levels

At least two dose levels are used including one showing signs of toxicity or reduced litter size. For non-toxic chemicals exposure to the maximum practicable dose should be used.

Procedure

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 dbserved. These days correspond to 7,25 , 8,25 and 9,25 days after conception. Successive treatments over these days may be used.

Analysis

The offspring 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-ventralline which are presumed to result from cell killing (WMVS);

(b) yellow, agouti-like, spots associated with mammae, genitalia, throat, axillary and inguinal areas and on the 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 solved by fluorescence microscopy of sample hairs.

Obvious gross morphological abnormalities of the offspring should be noted.

2. DATA

The data are presented as the total number of offspring scored and the number having one or more presumed somatic mutation spots. Treatment and negative control data are compared by appropriate methods. Data are also presented on a per-litter basis.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain 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 of which treatment was given,

-the route of treatment,

-the total number of offspring scored, and the number with WMVS, MDS and RS in the experimental and control groups,

-gross morphological abnormalities,

-Dose/response relationship of RS when possible,

-statistical evaluation,

-discussion of results,

-interpretation of results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.