#350-0

PROTOCOL FOR

DOMINANT LETHAL (MUTAGENIC) STUDY

SWISS WHITE MICE

I. Introduction

The mutagenic properties of a compound can be ascribed to its ability to produce subtle changes in the mammalian system, particularly changes in germinal cells of animals which may lead to morphological, physiological, and/or biochemical alterations in succeeding generations.

One means of detecting mutagenic responses in mammals is to measure the induction of dominant lethal mutations in the mouse. In this method, male animals are treated with a large single dose of the compound, and effects upon developing germinal cells are then monitored by mating the treated animal with non-treated females for six consecutive weeks. The females are then sacrificed, and the uterus and ovaries examined for the presence of implantations showing an early arrest in development. An increase in this parameter would be indicative of the induction of a mutagenic effect.

II. Procedure

Dose levels for administration are selected by conducting acute studies to determine the oral LD_{50}. Based on these data, maximum

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tolerated doses are ascertained and the dose levels employed are based on these figures.

In each experimental group, 10 male mice receive their respective dose and are subsequently mated with four virgin females. At the end of one full week, all females are removed and another group of four animals are placed in the mating cage. This is continued for six consecutive weeks, a period of time which covers spermatogenesis from the primary spermatocyte to the mature spermatozoon in the mouse. Work conducted at this laboratory has shown that most mutagenic compounds affect germinal cells in the post-meiotic stages, prior to or during the spermatid stage, and recovery from mutagenic effects is seen generally within three weeks after treatment. Since the germinal cells can be altered at any time during spermatogenesis, the evaluation of chromosomal damage should be monitored by extending the mating trials a full six weeks.

Females which were mated with treated males are then sacrificed one week after removal from the breeding cage. At that time, most of the animals are at mid-term of pregnancy. Sacrifice is conducted by an overdose of ether, and all animals are examined for signs of pregnancy. The corpora lutea, implantation sites and resorption sites are examined and numbers recorded. Resorption sites are divided into two groups, early deaths (deciduomata) and later deaths.

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The first, deciduomata, occurs at the site of the implanted blastocyte which fails to develop following implantation. The second is a lethal embryo which develops into a relatively advanced stage prior to death, so that the placenta and fetal membranes or remnants thereof are visible. The frequency of the latter is apparently unaffected by mutagens and, therefore, would appear to be non-genetic in this test system. The mutagenicity of the chemical is then measured by the proportion of all implantations which are deciduomata. In normal control matings the spontaneous incidence ranges from 4 to 6%, so that a positive effect is elicited by a compound showing a doubling of this spontaneous mutation rate.

III. Reports

At the conclusion of the entire study, a complete, formal report including an evaluation of the statistical significance of the findings, is submitted.

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