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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: William Miller
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THRU: Christine F. Chaisson, Ph.D. C.F. Chaisson
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Hazard Evaluation Division (TS-769)

SUBJECT: Review of Mutagenicity Assays with Rotenone.
EPA Reg. No. 6704-Q. Acc. No. 246587. Tox. No. 725.

Action requested: Review of 3 mutagenicity assays:

1. Rat bone-marrow cytogenetics assay
2. Mouse micronucleus assay.
3. Drosophila sex chromosome loss assay.

Background

In a meeting held on November 3, 1980 with representatives of the Fish and Wildlife Service and members of the Registration, Special Pesticide Review, and Hazard Evaluation Divisions, a list of proposed mutagenicity studies was presented for comment. The protocols were described, according to the minutes of the meeting, as adequate with respect to questions on mutagenicity of rotenone. The studies which are described in the attached review were on the list of protocols presented to the Agency during the meeting.

Conclusions

Because of deficiencies in conduct and design of the studies (see attached review), their results are unacceptable.

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Review

Citation: Biotech Research Laboratory, Inc., July 20, 1981. Analytical. Studies for the Detection of Chromosomal Abberrations in Fruit Flies, Rats, Mice, and Horsebeans. EPA Acc. No. 246587.

Three experiments were conducted with rotenene which was provided by the Fish and Wildlife Service, National Fishery Research laboratory, U.S. Department of the Interior. The test material was determined by the FWS to be 98% or greater according to the authors.

Experiment 1: In vivo cytogenetics study in rats.

This study was conducted in 8 to 10 week old male Sprague-Dawley rats. The test material was administered by oral gavage in corn oil. A preliminary toxicity study was conducted with groups of 20 rats given single doses of 50, 100, 200, or 300 mg per kg body weight. Based on mortality during a 14-day observation period following treatment, doses for the main study were selected. The doses selected for testing were 0, 0.7, 2.5, or 7.0 mg/kg. No information on the dosage schedule (whether single or repeated administration) was reported. Trimethylenemelamine (TEM) was used as the positive control substance.

The authors did not mention treating test animals with colchicine or colcamid prior to sacrifice. Use of this inhibitor of cell division is a standard procedure in such studies. There was also no mention of the sacrifice schedule for this experiment. Brusick, 1980; and A. L. Vans, 1976).

In view of the omissions of dosage and sacrifice schedules and of reporting use of a cell division inhibitor in test animals prior to their sacrifice, interpretation of reported results is not possible.

Experiment 2: Mouse micronucleus test.

In this experiment male and female ICR Swiss mice were given doses of rotenone in corn oil by gavage. Based on results from a preliminary acute toxicity study doses for the main study were chosen. These doses were 0, 10, or 80 mg/kg. The positive control group was given 1.0 mg TEM per kg body weight. A complete dosage regimen was not reported since the authors did not state whether they administered single or multiple doses. There was also only one sacrifice of animals at an unspecified time after dosing. These deficiencies preclude interpretation of reported results.

2

Experiment 3: Sex chromosome loss in Drosophila

Males of the F125, B/y^+ strain of D. Melanogaster were used.

The test material was first discolved in acetone, and the solution was then diluted with water so the final concentration of acetone was 0.2% or less. This solution, which contained appropriate concentrations of rotenone, was incorporated into the sucrose-agar medium. The positive control substance was methane sulfonate (MMS) in a sintered glass filter. Flies were exposed to the rotenone medium for 24 hours, while those treated with MMS were exposed for 2 days.

Concentration used in this experiment were 0, 1.0, 3.0, or 10.0 mM. After exposure the treated males were mated with virgin d 63 inscy females (one pair to each 2.5X5.0 mm vial containing 1 ml of sucrose-agar medium).

Mating parameters are as follows:

Group	Number mated	Number with progeny
Negative control	90	37
low dose	91	20
mid dose	91	18
high dose	80	15
MMS	84	37

These data indicated a low fertility rate in all groups. The number of progeny from successful matings was reported as follows:

Group	Males	Females	Average No. offspring per parent
Negative control	736	736	39.8
Low dose	363	383	37.4
Mid dose	395	367	42.3
High dose	747	797	41.7
MMS	301	308	40.6

3

The normal phenotype of male progeny was normal eyes and apricot body, and that for females was bar-eyes and yellow body according to the authors. The phenotypes used to indicate sex chromosome loss were normal eyes and yellow body in males and bar eyes and aprricot body. Chemically induced as well as spontaneous nondisjunction are represented by the same phenotype in males so the incidenceeof females with bar eye and apricot body resulting from paternal non-disjunction is used to correct for spontaneous nondisjunctions that might occur.

The author reported on 1 incidence of nondisjunction in each of the low and mid dose groups' progeny. None were noted in the high dose and positive control groups.

This assay is limited in scope when compared with a protocol recommended by Brusick (1980). In that protocol evaluation of at least 5000 progeny from each group, mating of at least 100 males according to a mass mating scheme, and use of tetraethyleneimino-1,4-benzoquinone as a positive control substance are the three primary differences. The variation in numbers of progeny scored in each group is large with some groups being twice as large as others, and the low fertility reported by the authors suggests that the study is not well conducted. A larger number of progeny would enable a more appropriate interpretation of the chromosome loss observed in the low and mid dose groups. Since the negative control group is twice the size of either the low or mid dose groups, the apparent significance of the reported results is considerable. In addition, the absence of a response in the positive control group suggests that MMS is an inappropriate choice for a reference substance, or the assay is not sensitive.

In view of these considerations the results of this study are unacceptable.

References

Brunsick., D. 1980. Principles of Genetic Toxicology. Plenum Press, New York.

Evans, H. J. 1976. Cytological methods for detecting chemical mutagens. Ch. 35. In. Hollaender, A. Chemical Mutagens: Principles and Methods for their Detection. Plenum Press, New York. pp. 1-30.

4