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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

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MEMORANDUM

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

Subject: Vitavax: ID Number 400-81  
Record Number 246480  
Tox Chem No. 165A  
HED Project No. 9-1616

From: John H.S. Chen, D.V.M. *John H.S. Chen 2/26/90*  
Review Section I  
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Thru: Yiannakis M. Ioannou, Ph.D., Section Head *M. Ioannou 2/27/90*  
Review Section I  
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and

Marcia Van Gemert, Ph.D., Branch Chief *M. Van Gemert 2/27/90*  
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Resistant: Uniroyal Chemical Company, Inc.  
Bethany, Connecticut 06525

Action Requested: Review and Assessment of Clastogenicity study  
with Vitavax in Rat Bone Marrow (Achikari, N.  
et al., Environmental and Molecular Mutagenesis  
12: 235-242, 1988)

Reviewer's Recommendation: The study was not properly performed  
to meet the minimum acceptability level for conducting the mammalian  
in vivo cytogenetic assay as recommended by EPA (References: EPA  
Health Effects Test Guidelines 560/6-83-001; Preston, R.J. et al.,  
Mutation Res. 87: 143-188, 1981). Therefore, the study is un-  
acceptable under the conditions tested.

*10/27/90*

Published Study: Genotoxic Effects of Some Systemic Pesticides including Vitavax: In Vivo Chromosomal Aberrations in Bone Marrow Cells in Rats.  
(N. Achikari and I.S. Grover; Environmental and Molecular Mutagenesis  
12: 235-242, 1988)

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### I. Materials and Methods:

1. Test Material: Vitavax is used against smuts and bunts and its annual consumption is about 80 mt. in India.

2. Test animals: Random-bred male albino rats of Wistar strain weighing 100-125 g and procured from Animal House, Haryana Agricultural University, Hissar, India.

3. Dose Determination: Toxicologic information regarding the LD50 for rats (3820 mg/kg/day) was used to prepare 3 doses of Vitavax (1/10 LD50 = 382 mg/kg/day; 1/20 LD50 = 191 mg/kg/day; 1/40 LD50 = 95.5 mg/kg/day) (See Reference: Kierthing, C.R. (ed) 1979: "The Pesticide Manual, A world Compendium", The British Crop Protection Council, Croydon).

4. Test Procedure and Administration: A volume of 0.2 ml of each concentration of Vitavax, dissolved in distilled water or suspended in pure DMSO, was administered intraperitoneally twice at interval of 24 hr. For the negative controls and positive control intraperitoneal injection of these controls of same volume as of Vitavax was administered. The rats were sacrificed 6 hr after the administration of last dose. EMS (62.5, 125, & 250 mg/kg) was used as the positive control for this study.

5. Preparation of the Bone Marrow: About 2 hours prior to sacrifice, the animals were intraperitoneally injected with 4 mg colchicine/kg. Bone Marrow cells were aspirated from femurs into normal physiologic saline. Following centrifugation to pellet the cells, the resultant pellets were resuspended in 1% sodium citrate. After 30 minutes of pretreatment at 37 C, the cells were resuspended in chilled Carnoy's fixative (3:1, methanol: acetic acid) for 2 hours. The cells again were centrifuged and resuspended in the same fixative for 24 hours. The fixed cells were dropped on chilled slides, which were air dried, stained with 10% Giemsa.

6. Statistical Evaluation: Approximately 40-50 well-spread metaphases per animal were scored from coded slides. The frequencies were checked statistically by  $2 \times 2 \chi^2$  contingency test. The results from control animals were checked for homogeneity by applying the  $\chi^2$  test and pooled as the samples were homogeneous. Gaps, pulverization, polyploidy and despiralization were excluded from the analysis of aberrant cell frequency.

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## II. Reported Results:

1. The positive control, EMS (62.5, 125, & 250 mg/kg), induced statistically dose-related increases in the percentage of aberrant cells ( $P < 0.05$ ; Table II attached).
2. The medium (125 mg/kg) and highest (250 mg/kg) doses of vitavax also causes statistically dose-related increases in the percentage of aberrant cells ( $P < 0.05$ ; Table II). The aberrants encountered frequently were chromatid fragments, ring chromosomes, chromatid breaks, and chromosome fragments. Micentricis encountered rarely. The breakage frequencies ranged from 0.05 to 0.21.

## III. Authors' Conclusion:

From the page 239 "Only the highest and the medium doses were found to be effective. The effect was dose dependent, with the frequencies of aberrant cells ranging from 4.8 to 16.9%."

## IV. Reviewer's Discussion and Conclusion:

1. The designed procedures for route of administration, treatment with colchicine, and chromosome preparation appear adequate for this experimental study. In addition, the positive control, EMS, adequately demonstrated the sensitivity of the rat bone marrow system to detect a clastogenic effect.
2. However, the clastogenicity of Vitavax in the rat bone marrow cytogenetic assay cannot be properly evaluated for this study due to the following deficiencies:
  - a) The critical information on the purity, batch number and contaminants of the test material was not provided.
  - b) The number and sex of animals used were inadequate. At least five males and five females per group should be employed for the rat bone marrow cytogenetic assay.
  - c) No toxicity data (either clinical toxicity or target cell cytotoxicity for test animals) were included in the test report. The mitotic index, which reflects cytotoxicity effect of the test material in the test animals, was also not given.
  - d) Details of type of number of aberrations given separately for each treated and control animal were not presented.

e) Although a brief description for dose determination (LD50) was included in this report, the LD50 (3820 mg/kg) for male albino rats used in this study was slightly lower than that (3956 mg/kg) obtained from the acute toxicity study with Vitavax in Sprague-Dawley CD male rats performed by Hazleton Laboratories (See Toxicology Branch MEMO to H. Jachy, 11/19/85, A. Arce and C. Skinner). Furthermore, there was no evidence of any toxic effect to test animals at 1000 mg/kg of Vitavax according to the Hazleton's test report from a repeated treatment schedule (five oral administrations). Therefore, it is questionable whether the highest dose (382 mg/kg) was appropriately chosen in this study (two IP administrations). It should be also noted that contaminants present in the test material could be responsible for the positive results in this study. The Authors did not clarify the possible contribution of contaminants to the positive responses.

f) Slides were not coded to eliminate bias prior to scoring and no analytical data were provided to support the actual concentrations used in the study.

g) Since different chemicals produce effects at different parts of cell cycle and cause different amounts of mitotic delay, multiple post-treatment sampling times should be used in this study. These sampling times (starting not earlier than 12 hrs after the last treatment and at appropriate intervals following the first sample but not extending beyond 72 hrs) will allow cells exposed in all stages of cell cycle to be analyzed.

h) The required statements of GLPs and OAU were not included.

3. Therefore, this study is judged to be inconclusive in the present form. The authors' conclusions of this rat bone marrow cytogenetic assay with Vitavax are not adequately supported by the data presented.

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