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2/8/93

DATA EVALUATION REPORT

CHEMICAL: Silver Zinc Zeolite (AgZn Zeolite) TOX. CHEM. NO.: N/A

STUDY TYPE: In vivo chromosome aberration assay in Sprague-Dawley rats

MRID NUMBER: 420328-04

SYNONYMS/CAS NO.: N/A

SPONSOR: Kanebo Zeolite USA, Inc.
New York, NY 10118

TESTING FACILITY: Arthur D. Little, Inc.
30 Memorial Drive
Cambridge, MA 02142

TITLE OF REPORT: Silver Zinc Zeolite-In vivo Chromosomal Aberration Assay in Sprague-Dawley Rats

AUTHOR(S): Kenneth S. Loveday

STUDY NUMBER(S): ADL 66365-00

REPORT ISSUED: May 13, 1991

CONCLUSION(S): Under the conditions of this study, Silver Zinc Zeolite did not induce chromosomal aberrations in bone marrow cells of male or female Sprague-Dawley rats following oral gavage at doses of 500, 1500 or 5000 mg/kg, and sacrifice times of 6, 18 or 24 hours after treatment.

CLASSIFICATION: Not Acceptable. The classification of this study is upgradeable to acceptable provided adequate explanation and clarification is given (on an individual animal basis) as to why slides could not be read from many of the males, particularly those of the 5000 mg/kg dosage group sacrificed at 18 hours.

A. Materials:

1. Test Material:
   Name: Silver Zinc Zeolite (AgZn Zeolite)
   Description: Solid, Powder
   Color: White
   Lot #: not given, received on June 13, 1989
   Purity: 99%, Ag (3.1%), Zn (6.1%), as supplied by sponsor
   Contaminants: not reported
   Solubility: Not soluble, suspended in 0.5% carboxymethyl cellulose (CMC) in deionized water

2. Controls:
   Negative: 0.5% CMC by oral gavage in deionized water (Sigma Chemical Company, lot number 114F-0414)

   Positive: Cyclophosphamide (30 mg/kg by oral gavage in deionized water) (Sigma Chemical Company, lot number 114F-0393)

3. Test compound:
   Volume of test substance administered: variable - volume of a 50, 150 or 500 mg/ml stock suspension required to give a dose of 500, 1500 or 5000 mg/kg (3.2 ml for heaviest rat)

   Route of administration: oral gavage

   Dose levels used: 500, 1500, 5000 mg/kg

4. Test animal:
   a. Species: Rat Strain: Sprague-Dawley Age: 8 - 10 wks
   Weight: male 235-320 g female 171-249 g
   Source: Taconic (Germantown, NY)

   b. No. animals used per dose: 5 + 1 males 5 + 1 females

   c. Properly maintained?
   YES

B. TEST PERFORMANCE

1. Treatment and Sampling times:
   a. Test compound
   Dosing: X once ___ twice (24 hr apart)
   ___ other (describe):

   Sampling (after last dose): X 6 hr ___ 12 hr X 24 hr
   ___ 48 hr ___ 72 hr (mark all that are appropriate)
   X other (describe): 18 hr

b. Negative and/or vehicle control

Dosing: _X_ once ___ twice (24 hr apart)
___ other (describe):

Sampling (after last dose): _X_ 6 hr ___ 12 hr _X_ 24 hr
___ 48 hr ___ 72 hr (mark all that are appropriate)
_X_ other (describe): 18 hr

c. Positive control

Dosing: _X_ once ___ twice (24 hr apart)
___ other (describe):

Sampling (after last dose): ___ 6 hr ___ 12 hr _X_ 24 hr
___ 48 hr ___ 72 hr (mark all that are appropriate)
___ other (describe):

2. Tissues and Cells Examined:

_X_ bone marrow ___ other (list):

3. Details of Slide Preparation:

Bone marrow cells were centrifuged, collected and resuspended in hypotonic buffer solution for 20 minutes at 37° C, then fixed 3 - 4 times in absolute methanol:glacial acetic acid (3:1) solution by centrifugation and resuspension. Drops of the concentrated cell suspension were placed on clean moist glass slides, air dried at least 24 hours and stained with 5% Giemsa for 5-8 minutes at room temperature.

4. Preliminary Cytotoxicity Assay:

No toxicity was observed in Sprague-Dawley rats following oral exposure to 5000 mg/kg of AgZn zeolite.

5. Cytogenetic Assay:

Six independent assays were done, three in females and three in males with sacrifice times as given above. In addition, a negative control group was run with all assays and a positive control group was included in the two 24-hour assays.

Structural chromosomal damage was evaluated in bone marrow cells arrested at the first metaphase following treatment with the test agent. Metaphase arrest was induced by injecting the rats with 1.5 mg/kg colchicine about 2 - 2.5 hours before sacrifice. Rats were killed by CO_2 asphyxiation and bone marrow cells collected from the femur(s) by flushing the cavity with 37° C hypotonic buffer solution (0.03 M KCl, 0.01 M sodium citrate) using a hypodermic needle and a syringe.

The percent mitotic index (MI) on an individual animal basis was calculated by counting the number of metaphase cells in at least 500 cells. %MI = (# metaphase cells observed / total # cells observed) x 100.

Cells were analyzed for chromatid and chromosome breaks, chromatid and chromosome gaps, interstitial deletions, double minute chromosomes, dicentrics, ring chromosomes, triradials, quadriradials, complex rearrangements, cells with at least one pulverized chromosome and cells with greater than 10 aberrations.

The total number of aberrations, the number of aberrations per cell, the number of cells with aberrations, and the percent of cells with aberrations were calculated twice, once including gaps in the calculations and once not including gaps. Normally, gaps are not included in calculating chromosomal aberrations. AgZn Zeolite did not induce an increase in chromosomal aberrations in either male or female rats as tested in this study either with or without gaps included in the calculation. No toxicity was observed during the study.

6. Reviewer's Discussion/conclusions:

The study followed the pertinent federal guidelines for conducting an in vivo chromosomal aberration assay in rat bone marrow cells (however, as discussed in the next paragraph, the number of male rats actually analyzed falls short, in many cases, of the desired five animals). The results were consistently negative in both male and female rats at all tested concentrations and sampling times. The authors of the study state that the number of chromosomal breaks and gaps were too low in both the control and test animals to evaluate using a quantitative statistical method; therefore, "the frequency of cells with chromosomal damage from the negative control groups were pooled from male and female animals to provide a range of values." Frequencies of aberrant cells from animals exposed to AgZn zeolite were compared to this range.

The authors state that six animals were dosed per test group (13 test groups per sex) and that 50 metaphase cells from each of five animals were analyzed for chromosomal aberrations where possible. This was possible in 11 of the 13 female groups but in only five of the 13 male groups (see attached tables 7 and 14). Three rats were analyzed in six of the male groups and only one rat in the 18-hour 5000 mg/kg group. The authors do not comment on this difference. Reasons given for not analyzing a slide included poor quality metaphase cells or low mitotic index. Why this appears to be predominantly a problem with males is not discussed. No toxicity was observed as a result of AgZn zeolite exposure although two male rats died as a result of gavage errors, one from the six-hour group and one from the 18-hour group. The rat from the 18-hour group was replaced with an extra rat. Therefore, the difference was not a smaller number of male rats to analyze.

Results from positive and negative controls were as expected. Summary tables of results for male and female rats are attached.

In conclusion, this study will meet the requirements for an in vivo cytogenetics assay in rats if an explanation is given for the smaller number of male rats analyzed.

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7. Was the test performed under GLPs (is a quality assurance statement present)?
YES

8. CBI appendix attached?
NO

Silver Zinc Zeolite OER

Page ___ is not included in this copy.

Pages 6 through 8 are not included in this copy.

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