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Guideline Series 84: **MUTAGENICITY**

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DATA EVALUATION REPORT

CHEMICAL: Silver Copper Zeolite Tox. Chem. No.: 129057

STUDY TYPE: In vivo mammalian cytogenetics assay in Sprague-Dawley rats (84-2)

ACCESSION or MRID NUMBER: 416158-10

HED NO.: 1-0266

SPONSOR: Kanebo Zeolite U.S.A., Inc.; 20th Fl., Empire State Bldg.; 350 5th Ave.; NY, NY 10118

TESTING FACILITY: Arthur D. Little, Inc.; Acorn Park Cambridge, MA 02140

TITLE OF REPORT: Silver Copper Zeolite - In Vivo Chromosome Aberration Assay in Sprague-Dawley Rats

AUTHOR(S): Kenneth S. Loveday, Ph.D.

STUDY NUMBER(S): 63613-23 (ADL #)

REPORT ISSUED: April 24, 1990

GLP COMPLIANCE: Statement included.

QUALITY ASSURANCE: Statement included.

CONCLUSION(S) - Executive Summary:

The administration of Silver Copper Zeolite by oral gavage did not induce a significant increase in chromosomal aberration in the male and female rats at the 6-, 18- and 24-hour exposure periods. It was, therefore, considered to be non-clastogenic in Sprague-Dawley rats at the dose levels tested (500, 1500 and 5000 mg/kg).

This study satisfies requirements according to guidelines (84-2) for an in vivo mutagenicity study.

Classification: Acceptable

A. MATERIALS

1. Test Material: Name: Silver Copper Zeolite
Description (e.g. technical, nature, color, stability):
solid; light blue powder
Batch #: not given Purity: 99%
Contaminants: not reported

2. Control Materials:
Negative (if not vehicle)/Route of administration:
0.5% Carboxymethyl cellulose, Lot No.: 114F-0414 (Sigma Chemical Co. Dosed at 10 ml/kg./ Oral gavage

Positive/Final dose(s)/Route of administration:
Cyclophosphamide, Lot No.: 33F-0157 (Sigma Chemical Co.)
Dosed at 30 mg/kg

3. Test compound:
Volume of test substance administered:
10 ml
Route of administration:
oral gavage
Dose levels used:
500, 1500 or 5000 mg/kg

4. Test animals:
a. Species rat Strain Sprague-Dawley Age 8-9 weeks
Weight male 248-365 g female 162-203 g
Source: Taconic; Germantown, NY

b. No. animals used per dose: 5 males 5 females
c. Properly maintained? Yes

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
a. Test compound: Silver Copper Zeolite
Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): X 6 hr X 18 hr
X 24 hr _____ 48 hr _____ 72 hr

b. Negative and/or vehicle control
Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): X 6 hr X 18 hr
X 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)

_____ other (describe):

c. Positive control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

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

d. Administration of spindle inhibitor
Inhibitor used/dose:
Colchicine 1.5 mg/kg (Due to a technical error, the 6-hour group was given between 2.2-2.3 mg/kg.
Interval administered before animal killed:
2 1/2 hours prior
Route of administration: __X__ i.p. _____ other (describe)

2. Tissues and Cells Examined:

__X__ bone marrow _____ other (list):

No. of cells per animal per treatment group examined:
500 spreads/treatment group; 50 spreads/animal; 250 spreads/sex
No. of cells per animal per control group examined:
500 spreads/treatment group; 50 spreads/animal; 250 spreads/sex

3. Details of cell harvest and slide preparation (if appropriate, attach copy of procedures):

Test animals were injected with 1.5 mg/kg colchicine intraperitoneally approximately 2 - 2.5 hour prior to sacrifice. Males in the 6-hour group were accidentally injected with 2.2 - 2.3 mg/kg colchicine. However there was no change in the outcome of results since the chromosomal spreads were suitable for analysis. After the rats were sacrificed bone marrow cells were collected from the femurs of each animal using a prewarmed hypotonic buffer solution (0.03 M KCL, 0.01 M Na citrate). They were centrifuged, collected and resuspended in hypotonic solution for 20 minutes at room temperature. Cells were then fixed 3-4 times in methanol:acetic acid (3:1) by centrifugation and resuspension. They were then dropped on clean glass slides, air dried and stained with 5% Giemsa stain for about 5 minutes.

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Metaphase cells were analyzed for chromosomal aberrations through a high power lens (100X oil). The mitotic index was determined by counting at least 500 cells. Fifty metaphase cells from each of 5 animals/test condition were analyzed where possible. Metaphase cells were analyzed for the following types of chromosomal aberrations:

chromatid gap
chromatid break
double minute chromosomes
interstitial deletions
complex rearrangements
cells with at least one pulverized chromosome
rings
triradial
quadriradial
chromosome gap
chromosome break
dicentric chromosome
cells with greater than 10 aberrations

4. Quantitation and Analysis of Data

The animals weights, number of cells analyzed, mitotic indices and the type and number of aberrations were recorded. The percent mitotic index was determined by counting a minimum of 500 cells.

Two methods were used to calculate the total number of aberrations, the number of aberrations per cell and the percent cells with aberrations. In one calculation, the chromatid and chromosome gaps were counted as aberrations and in the other, the gaps were not counted as aberrations. The number of cells with at least one aberration was divided by the total number of metaphase cells, at each dose level, to determine the percentage of cells with aberrations.

5. Results:

Clinical observations:
During the 24-hour observation period, several males had either discoloration of the eyes, a blood spot under the ear or a bloody spot on the forehead. None of these findings were considered significant because no dose response was observed.

At 5000 mg/kg 2 females and 9 males were observed to have soft feces; and 2 females had urine stains. Theses effects may have been due to Silver Copper Zeolite

One male from the 6-hour 500 mg/kg dose group was found dead before colchicine was administered. Its death did not appear to the registrant to be due to test article administration.

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Chromosomal Aberration Assay:
In male rats, the percentages of cells with aberrations, excluding gaps, for the negative control values ranged from 0.0%-2.0% in the 6-, 18- and 24-hour assays (Tables 5, 7 and 9). When the percentages of cell with aberrations from those tested with Silver Copper Zeolite were compared to the negative control values, there was no significant increase in any of the exposure periods.

In the 24-hour assay for the positive control, there was a significant increase in the percentages of aberrant cells from 5/5 animals (Table 9).

Silver Copper Zeolite did not induce a statistically significant increase in chromosomal damage for the three assays in the male rats (Table 16).

The percentages of cells with aberrations excluding gaps, in the female rats, in the negative controls ranged from 0.0%-4.0% in the 6-, 18- and 24-hour assays (Tables 11, 13 and 15). The percentages of cells with aberrations for Silver Copper Zeolite were not significantly greater than the negative control values.

After gaps were added into the calculation, the percentages of aberrant cells from the negative control ranged from 0.0%-6.0% at 6-, 18- and 24-hours. The percentages of cells with aberrations from Silver Copper Zeolite were not significantly increased compared to the negative control value.

There was a significant increase in the percentages of aberrant cells in 5/5 animals in the positive control group (24-hour) as can been seen in Table 15 from the study.

A summary of results, as seen in Table 17 from the study, shows that Silver Copper Zeolite did not induce a significant increase in chromosomal aberration for the three exposure periods in female rats. We agree with the study Author's conclusion that Silver Copper Zeolite did not cause a significant increase in chromosomal aberration.

6. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

The administration of Silver Copper Zeolite by oral gavage did not induce a significant increase in chromosomal aberration in the male and female rats at the 6-, 18- and 24-hour exposure

periods. The rationales for acceptability of this study are given below:

a) The positive control (cyclophosphamide) adequately demonstrated the sensitivity of bone marrow cells to detect a clastogenic response; even at the highest dose level of test material (5000 mg/kg for both male and female rats), there was not a significant increase in chromosomal aberration observed.
b) The test material has been tested up to a maximum of 5000 mg/kg dose level.
c) Although the stability of the test material was not provided in this report, evidence of toxic effects of the test material at 5000 mg/kg in test animals was observed.
d) Based on the results reported in this study, no significant difference in mitotic index was found between the highest dose group and the corresponding control group. The test material did not cause any mitotic suppression in test animals (males or females). Therefore, the harvest times (6, 18,and 24 hours post-treatment) used in this study appear adequate for the detection of chromosomal aberrations in the bone marrow of Sprague-Dawley rats.
Based on these findings, we assess that the study was properly conducted and that the study Author's conclusion was correct. Silver copper zeolite is non-clastogenic in Sprague-Dawley rats.

This study satisfies requirements according to guidelines (84-2) for an <u>in vivo</u> mutagenicity study. It is classified acceptable.

Silver Lodge DEA

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