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Guideline Series 84: **MUTAGENICITY**

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DATA EVALUATION REPORT

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

HED NO.: 1-0266

STUDY TYPE: Mammalian cells in culture cytogenetics assay in Chinese hamster ovary) cells

ACCESSION or MRID NUMBER: 416158-09

SPONSOR: Kanebo Zeolite U.S.A., Inc.; 5th Ave., NY, NY 10118

TESTING FACILITY: Arthur D. Little; 30 Memorial Dr.;, Cambridge, MA 02142

TITLE OF REPORT: Silver Copper Zeolite: In Vitro Chromosomal Aberration Assay

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

ADL REF. NO.: 63613-22

REPORT ISSUED: July 11, 1990

GLP COMPLIANCE: Statement included on page 3.

QUALITY ASSURANCE: Statement included on page 6.

CONCLUSION(S) - Executive Summary:
Silver Copper Zeolite was tested for its ability to induce chromosomal damage in vitro using Chinese hamster ovary (CHO) cells using both activated and nonactivated assays. Although there was no increase in the percentage of aberrant cells in the non-activated assays, there was a significant increase in the 10-hour activated assays. At the 75, 100 and 125 ug/ml concentrations, during the 10-hour activated assay, there was a statistically significant increase in the percentage of aberrant cells.

This study satisfies requirements according to guidelines for a mutagenicity study (84-2) and is classified acceptable.

A. MATERIALS

1. Test Material: Name: Silver Copper Zeolite
   Description: light blue solid; powder

   Batch #: not specified   Purity: 99%
   Contaminants: not reported

2. Control Materials:
   Negative: McCoy's 5A medium; Lot #: Sigma (58F-4602-1)
   Solvent/final concentration:

   Positive: Non-activated (concentrations, solvent):
   Mitomycin C; Sigma Chem. Co.,Lot #: 123F-0463, slightly blue. (0.3 & 1 ug/ml in deionized water)

   Activated (concentrations, solvent):
   Cyclophosphamide; Sigma Chem. Co., Lot #: 33F-0157, white (50 ug/ml in deionized water)

3. Activation: S9 derived from

_X_ Aroclor 1254	_X_ induced	_X_ rat	_X_ liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

   If other, describe below
   Describe S9 mix composition (if purchased, give details):
4.5 mg/ml Isocitric acid; 2.4 mg/ml NADP; and 20 ul/ml S9 fraction Purchased from Microbiological Associates, Bethesda MD, Lot/Batch #411.

4. Test compound concentrations used:

Non-activated conditions:
10-hour assay: 100, 50, 30, 15, 5 & 1.5 ug/ml.
20-hour assay: 100, 50, 30, 10 & 3 ug/ml

Activated conditions:
10-hour assay: 500, 150, 100, 50, 30, 10 & 3 ug/ml
repeat 10-hour assay: 150, 125, 100, 75, 50, 25 and 10 ug/ml.

20-hour assay: 5000, 1500, 500, 150, 50, 15, & 5 ug/ml
repeat 20-hour assay: 150, 125, 100, 75, 50, 25 and 10 ug/ml

5. Test Cells: mammalian cells in culture
   Describe cell line, cell strain or primary cell culture (if human lymphocytes, describe conditions of subjects) used:

Frozen cultures of mycoplasma-free, cloned Chinese hamster ovary

(CHO) cells from the Merck Institute for Therapeutic Research, West Point, PA.

Cells were maintained in a 37 °C, 5% CO_2 and 95% relative humidity incubator in culture medium containing McCoy's 5A with $NaHCO_3$ buffer, 10% fetal bovine serum, 50 units/ml penicillin, 50 ug/ml streptomycin and 2 mM L-glutamine.

Properly maintained? Yes

Cell line or strain periodically checked for Mycoplasma contamination? Yes

Cell line or strain periodically checked for karyotype stability? not stated

B. TEST PERFORMANCE

1. Cell treatment:
   a. Cells exposed to test compound for:
      10 & 20 hours (non-activated) 10 & 20 hours (activated)
      For Activated Assay: 10 hrs = 2 hrs exposure time + 8 hrs growth time
   b. Cells exposed to positive controls for:
      10 & 20 hours (non-activated) 10 & 20 hours (activated)
      For Activated Assay: 20 hrs = 2 hrs exposure time + 18 hours growth time
   c. Cells exposed to negative and/or solvent controls for:
      10 & 20 hours (non-activated) 10 & 20 hours (activated)

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; if lymphocytes, nature of mitogen and when added; cell density during treatment; harvest times; spindle inhibitor and when used; chromosome preparation and analysis; number of cells/culture analyzed; statistics used):

Non-activated assay:
CHO cells were seeded at a density of 5 X 10^5 in 25 cm^2 tissue culture flasks approximately 24 hours before the start of the experiment. They were exposed to either the test substance/medium mixture, a positive or a negative control. Vinblastine sulfate (0.26 ug/ml) was added for 2 hours prior to harvest. After incubation, metaphase cells were collected by trypsinization and centrifugation of the cells. Phosphate buffer saline was used to wash the test substance and positive control still remaining in some flasks. A hypotonic solution (0.03 M KCL and 0.01 M sodium citrate) was used to suspend the harvested cells in for 12 minutes.

at 37° C. It was fixed with methanol:acetic acid (3:1). Drops of concentrated cell suspension were placed on glass slides, air dried and stained with 5% Giemsa. For the non-activated assay, two exposure periods were used, 10 and 20 hours.

Activated assay:
The methods used in the activated assay comply with the nonactivated assays with following exceptions:
Test sample concentrations tested in the 10-hour activated assay were 500, 150, 100, 50, 30, 15, 10, 5, 3, 1.5 and 1.0 ug/ml. In the repeat 10-hour assay the concentrations were 150, 125, 100, 75, 50, 25 and 10 ug/ml. Concentrations tested in the 20-hour assay were 5000, 1500, 500, 150, 50, 15, 5, 1.5,, 0.5 and 0.15 ug/ml. Concentrations in the repeat 20-hour assay were 150, 125, 100, 75, 50, 25, 10 ug/ml. Cells were exposed to the test sample and controls for 2 hours in serum free medium containing S9. After exposure, cells were washed with PBS and allowed to grow for 8 or 18 hours in serum supplemented medium. About 2 hours before cell harvesting 0.26 ug/ml of vinblastine sulfate was added. The use of these two assay times ensure the harvest of mitotic cells, even if cell cycle delay is caused by the chemical.

Metaphase cells were analyzed for chromosomal aberrations through a microscope. After counting a minimum of 500 cells, the mitotic index was determined. At least 25 cells were analyzed for the positive control and based on acceptable metaphase cells, a minimum of 100 cells from at least 3 test sample concentrations and the negative control 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

3. Quantitation and analysis of Data:

The data were analyzed statistically using Margolin et al (1986, "Statistical Analyses for *In Vitro* Cytogenetic Assays Using Chinese Hamster Ovary Cells."Environ. Mutagen 8:183-204.

4. Cytogenetics assay (reported results, e.g. induction of aberration frequency; types of aberrations, e.g. whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per

concentration; levels of cytotoxicity obtained, e.g. effect on mitotic index or cell survival, if examined; include representative table, if appropriate):

When tested in the nonactivated 10-hour assay, no metaphase cells were observed at 100 ug/ml due to toxicity. Low mitotic indices were observed at 50 ug/ml (0.2% and 0.4%) and 30 ug/ml (0.4% and 0.6%) as shown in Table 1 from the study. In the duplicate 10-hour nonactivated assay the three test sample concentrations analyzed were 15, 5 and 1.5 ug/ml. The percentages of aberrant cells in controls (Table 2) were 4% and 10%. There was no significant increase over background in the number of aberrant cells in the test groups.

Toxicity was observed at 100 ug/ml in the 20-hour nonactivated assay (Table 3. Four test sample concentrations, 50, 30, 10 and 3 ug/ml were analyzed; only cells from the 50 ug/ml flask were suitable for analysis (Table 3). In both solvent controls (Table 4) the percentage of aberrant cells was 3%. There was no statistically significant increase in the percentages of aberrant cells when compared to controls. We agree with the study Author's conclusion that silver copper zeolite did not cause an increase in chromosomal aberrations in the non-activated assays.

Activated assays of 10 and 20 hours were performed on Silver Copper Zeolite. At 500 ug/ml(Table 5), in the first 10-hour assay, the test sample was toxic. At 150 ug/ml, there was a low frequency of acceptable metaphase cells, therefore, this sample was not analyzed. Of the sample concentrations that were analyzed (100, 50, 30, 10 and 3 ug/ml) only cells from the 100 ug/ml flask were suitable for analysis. The percentages of aberrant cells in the duplicate controls were 8% and 18% (Table 6). There was a statistically significant increase in the percentage of aberrant cells to 30% at 100 ug/ml.

In the repeat 10-hour activated assay toxicity was again observed at 150 ug/ml. The test samples concentrations analyzed were 125, 100, 75, 50 and 10 ug/ml (Table 7). In the duplicated solvent controls the percentages of aberrant cells excluding gaps were 2% and 6%. A concentration-dependent increase in the average percentage of aberrant cells was observed (5%, 6%, 10%, 7% and 15%, respectively, for 10, 50, 75, 100 and 125 ug/ml). There was a statistically significant increase observed at 75 and 125 ug/ml.

Silver Copper Zeolite was toxic at 5000, 1500 and 500 ug/ml (Table 9) in the first 20-hour activated assay as no metaphase cells were observed. Cells were not analyzed at 150 ug/ml due to the low mitotic index and absence of metaphase cells. The percentage of aberrant cells in the duplicate solvent controls were 1% and 0% (Table 10). Of the test sample concentrations analyzed (50, 15 and 5 ug/ml) a statistically significant increase to 5.5% of aberrant

cells was observed at 50 ug/ml.

Of the six test sample concentrations analyzed (150, 125, 100, 75, 50 and 25 ug/ml) in the repeat 20-hour test, only cells in the 150 ug/ml flask were suitable for analysis due to a low mitotic index of 0.4% in the duplicate flask (Table 11). The percentage of aberrant cells in the duplicate solvent controls excluding gaps were 3% and 7% (Table 12). There was no significant increase in the percentage of aberrant cells in any of the test concentrations. Therefore, the small positive increase observed in the first 20-hour assay was not reproducible under the same experimental conditions. We agree with the study Authors conclusion that silver copper Zeolite induced significant increases in chromosomal aberrations in CHO cells in the 10-hour nonactivated assay at 75, 100 and 125 ug/ml.

Because the positive controls utilized in all assays produced a significant amount of chromosomal damage, the test system and the S9 were efficacious.

5. <u>Reviewer's discussion/conclusions</u> (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions; remember, do not include gaps in final aberration frequency analysis):

Silver Copper Zeolite was tested for its ability to induce chromosomal damage <u>in</u> <u>vitro</u> using Chinese hamster ovary (CHO) cells using both activated and nonactivated assays. Although there was no increase in the percentage of aberrant cells in the non-activated assays or the 20-hour assays, there was a significant increase in the 10-hour activated assays. The rationales for the acceptability of this study are given below:

a) The positive control compounds (MMC & CP) adequately demonstrated the sensitivity of the cultured CHO cells with or without metabolic activation to detect a clastogenic response;
b) The highest dose levels of test material (100 ug/ml -S9; 150 ug/ml +S9 demonstrated cytotoxicity to CHO cells, resulting in low frequency of acceptable metaphase cells);
c) Although the preliminary assessment of cell cycle delay was not conducted in this study, the two harvest times (10- & 20-hours posttreatment) for cells exposed to the test material in the presence or absence of metabolic activation appeared adequate for the detection of chromosomal aberration in cultured CHO cells.

Based on these findings, we assess that the study was properly conducted and the study Author's conclusions of the data was correct. Silver copper Zeolite is clastogenic in the activated assay only (10-hour assay; 75-125 ug/ml).

This study satisfies requirements according to guidelines for a

mutagenicity study (84-2) and is classified acceptable.

6. Was test performed under GLPs (is a quality assurance statement present)? Yes

7. CBI appendix attached No

Silver Zeolite, DER

Page ___ is not included in this copy.

Pages 8 through 19 are not included in this copy.

The material not included contains the following type of information:

- ___ Identity of product inert ingredients.
- ___ Identity of product inert impurities.
- ___ Description of the product manufacturing process.
- ___ Description of quality control procedures.
- ___ Identity of the source of product ingredients.
- ___ Sales or other commercial/financial information.
- ___ A draft product label.
- ___ The product confidential statement of formula.
- ___ Information about a pending registration action.
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