008642

5/16/1991

Guideline Series 84: **MUTAGENICITY**

Reviewed by: SanYvette Williams, D.V.M. SWW 5/6/91
Section IV, Tox. Branch II
Secondary reviewers: John Chen, D.V.M. John M Chen 5/14/91
Section I, Tox. Branch II
Elizabeth Doyle, Ph.D E A Doyle 5/16/91
Section IV, Tox. Branch II

DATA EVALUATION REPORT

CHEMICAL: Silver Copper Zeolite

TOX. CHEM. NO.: 129057 HED PROJECT NO.: 1-0266

STUDY TYPE: Salmonella/mammalian activation gene mutation assay (84-2)

ACCESSION: 416158-08

SPONSOR: Kanebo Zeolite USA; 20th Fl., Empire State Bld. 350 Fifth Ave., New York, NY 10118

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

TITLE OF REPORT: Silver Copper Zeolite - Ames/Salmonella Mutagenicity Assay

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

STUDY NUMBER(S): 63613-21

REPORT ISSUED: April 16, 1990

GLP COMPLIANCE: Signed and dated statement on page 3.

QUALITY ASSURANCE: Signed and dated statement on page 6.

CONCLUSION(S) - Executive Summary:
After a preliminary assay and three subsequent assays to determine whether Silver Copper Zeolite was able to induce mutations in Salmonella typhimurium in the presence and absence of a metabolic activation system, it was determined that this substance was not mutagenic to TA98, TA100, TA1535 and TA1537 strains of Salmonella typhimurium at the concentrations tested (0.005 to 0.15 mg/plate without activation; 0.005 to 1.5 mg/plate with activation.

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 (e.g. technical, nature, color, stability):
Blue solid, powder; not stable
Purity: 99% (Batch No. CV03129BV)
Contaminants: not reported
Solvent used: Sterile water
Other comments:

2. Control Materials:
Negative: Deionized water plus phosphate buffered saline for nonactivated assay
Deionized water plus S9 mix for Activated assay

Positive: Non-activation:
a) Sodium azide 10 ug/plate TA100
0.5 ug/plate TA1535; Sterile water is the solvent for both and each was in stock solution aliquots of 100 ul/plate.

b) 2-Nitrofluorene 3 ug/plate TA98; DMSO is the solvent. Stock solution aliquots of 100 ul/plate.

c) 9-Aminoacridine 80 ug/plate TA1537; DMSO is the solvent. Stock solution aliquots of 100 ul/plate.

Activation:
2-Aminoanthracene (2-anthramine) 0.5, 1, 2 and 3 ug/plate for strains TA98, TA100, TA1535 and TA1537, respectively.

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):

| | |
|---|---|
| 0.4M $MgCl_2$/1.65M KCL | 0.2 ml |
| 1M Glucose-6-Phosphate | 0.05 ml |
| 0.1M NADP | 0.4 ml |
| 0.2M Phosphate Buffer pH 7.4 | 5.0 ml |
| Sterile Deionized $H_2O$ | 3.35 ml |
| S9 Fraction | 1.0 ml |

4. Test organisms: S. typhimurium strains

___ TA97 _X_ TA98 _X_ TA100 ___ TA102 ___ TA104
_X_ TA1535 _X_ TA1537 ___ TA1538 ; list any others:
Properly maintained? YES
Checked for appropriate genetic markers (rfa mutation, R factor)? YES

5. Test compound concentrations used:
Non-activated conditions: Initial - 5, 1.5, 0.5, 0.15, 0.05 and 0.015 mg/plate.
Subsequent - 0.15, 0.1, 0.05, 0.03, 0.015, 0.010 and 0.005 mg/plate.
Activated conditions: 1.5, 0.5, 0.15, 0.05, 0.015 and 0.005 mg/plate.
3 replicates/concentration/strain

B. TEST PERFORMANCE

1. Type of Salmonella assay:
_X_ standard plate test
___ pre-incubation (___ minutes)
___ "Prival" modification (i.e. azo reduction method)
___ spot test
___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation):

The tests were carried out in accordance with the method described by Ames et al, (Mutation Res. 31: 347-364, 1975).
Four strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) were tested for crystal violet sensitivity caused by the rfa mutation. Ampicillin resistance was tested to verify the presence of the plasmid (R-factor) in TA98 and TA100. The other two do not have the R-factor. For each assay, each strain displayed the proper genetic characteristics.

Silver Copper Zeolite was tested in initial activated and nonactivated assays of all four strains at 5, 1.5, 0.5, 0.15, 0.05 and 0.015 mg/plate using sterile water as the suspension vehicle. Excessive toxicity in the nonactivated assay led to a reduction in concentration of subsequent assays: 0.15, 0.1, 0.05, 0.03, 0.015, 0.010 and 0.005 mg/plate. Negative and positive controls were tested with each strain for each assay, and all conditions were plated in triplicate.

Also added were 2.5 ml aliquots of molten top agar containing 50 uM biotin and 50 uM histidine: 100 ul of bacterial culture with a density between 1 and 2 x $10^9$ bacteria/ml, 100 ul of the

appropriate concentration of the test substance or positive or negative control, and 500 ul of S9 mix or buffer (for the nonactivated assay). The tubes were mixed and poured onto plates containing 25 ml of minimal glucose agar. They were incubated at 37°C for approximately 72 hours. After this time they were counted. A substance to be characterized as positive in the Ames test has to fulfill the following requirements: a) at least doubling of the spontaneous mutation rate, and b) dose-response relationship.

2. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

In the preliminary cytotoxicity test (Tables 1 & 2 attached), Silver Copper Zeolite was tested in initial activated and nonactivated assays at 5, 1.5, 0.5, 0.15, 0.05 and 0.015 mg/plate using sterile water as the suspension vehicle. Excessive toxicity to the bacteria was observed at 5, 1.5 and 0.15 mg/plate. Thus, the concentrations were reduced from 0.15 to 0.005 mg/plate for the non-activated test.

Although there was no dose-response relationship, the only significant increase in the number of revertant colonies/plate seen in the nonactivated assay was at the 0.05 mg/plate concentration in strain TA1537 (49 revertants/plate). There was also a 2-fold increase at 0.05 mg/plate in strain TA1535 (29 revertants/plate vs 10/plate in the negative control). In the activated assay there was no significant increase in the revertants/plate for any of the four strains.

3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The concentrations of this assay were reduced after the preliminary study. The highest concentrations were 0.15 mg/plate in The non-activated assay (Table 3 excerpted from study). To determine reproducibility, intermediate concentrations were added to the nonactivated assay to see if the revertants in strain TA1537 increased again. Strain TA1535 was also retested because the revertants at 0.05 mg/plate was close to the significant level. In the nonactivated assay, toxicity was observed at 0.15 and 0.10 mg/plate for all four strains while strain-specific toxicity was

observed at 0.05 and 0.03 mg/plate. Unlike the first nonactivated assay, there was no significant increases in the number of revertants colonies/plate for any of the strains. There were inconsistencies in data for strain TA98, therefore it was not evaluated. Results of the second mutagenesis assay showed that no positive mutagenic responses were observed with strains TA100, TA1535 and TA1537 with or without metabolic activation

A third nonactivated mutagenesis assay was performed on strains TA98, TA1535 and TA1537. Silver Copper Zeolite was toxic at 0.15, 0.10 and 0.05 mg/plate (Table 5 excerpted from study). There was no significant increase in the number of revertants/plate for any strain in this experiment. Therefore, the increase in revertant colonies seen in Table 1 for strains TA1535 and 1537 was not reproducible in two subsequent experiments. Results of the third mutagenesis assay showed that Silver Copper Zeolite was non-mutagenic to strains TA98, TA100 and TA1537 with or without metabolic activation at any concentrations tested. (Table 5)

4. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate.

The spontaneous revertant colonies for each of the four tester strains of Salmonella typhimurium are found within normal ranges of revertant colonies recommended by the Salmonella/mammalian-microsome mutagenicity test (Ames et al., Mutation Res. 31: 347-364, 1975).

The strain specific control compounds (sodium azide, 2-nitrofluorene and 9-aminoacridine) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significant positive responses over the negative control values. These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation to detect chemical mutagens.

No statistically significant increases (less than 2-fold) in the number of revertant colonies for any of the four tester strains were observed following exposure to the test material either in the presence or absence of metabolic activation (Tables 1-6). Therefore, the test compound, Silver Copper Zeolite, was nonmutagenic to TA98, TA100, TA1535 and TA1537 strains of Salmonella typhimurium in the salmonella/mammalian-microsome mutagenicity test.

Silver Zeolite OER

---

Page ___ is not included in this copy.

Pages 6 through 10 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.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

---

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

---