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GUIDELINE: 83-3

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rats
Guideline: #83-3

EPA Identification No.s: EPA MRID (Accession) No.: 416385-02
Tox. Chemical No.: 129057
HED Project No.: 1-0266

Test Material: Silver Copper Zeolite

Sponsor: Kanebo Zeolite USA, Inc., 350 5th Ave., NY, NY 10118

Testing Facility: Arthur D. Little, Inc., 30 Memorial Dr.,
Cambridge, Massachusetts 02142

Title of Report: Study of Teratology in Pregnant Rats
Administered Silver-Copper Zeolite Orally (#83-3)

ADL Reference No.: 63613-18

Author(s): Judith Marquis, Ph.D., D.A.B.T.

Report Issued: 1 August 1990

Conclusions: Maternal toxicity was evidenced at 2000 mg/kg by reduced body weight gain. Daily observations also indicated that silver copper zeolite was having an effect on the high dose animals. Developmental toxicity was observed at 2000 mg/kg as skeletal variations.

Core Classification: Supplementary
This study does not satisfy guideline requirements (83-3) for a developmental toxicity study. It can be upgraded with the submission of the historical control data.

Maternal NOEL = 700 mg/kg
Maternal LOEL = 2000 mg/kg based on reduced body weight gain.
Developmental Toxicity NOEL = 700 mg/kg
Developmental Toxicity LOEL = 2000 mg/kg based on abnormal

fetuses from a dam that exhibited signs of maternal toxicity.

A. MATERIALS

Test Compound: Purity: 99%
Description: light blue powder
Lot No.: not included
Contaminant: list in CBI appendix not included

Vehicle(s): 0.5% carboxymethylcellulose (Sigma Chemical Co., lot no. 67F-0526)

Test Animal(s): Species: rat
Strain: Sprague-Dawley
Source: Taconic Farms, Germantown, NY
Age: adult, pre-mated females
Weight: Batch 1 - 200-293 g
Batch 2 - 211-259 g

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of Silver Copper Zeolite when administered by oral gavage to pregnant rats on gestation days 6 through 15, inclusive. Pregnant rats were received on gestation day 2 according to Dr. Loveday of Kanebo Zeolite on July 31, 1991.

1. Dosing:

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	200	30
Mid Dose	700	30
High Dose	2000	30

All doses were in a volume of 1 ml/0.1 kg of body weight/day prepared weekly during the dosing period. The dosing solution was analyzed quantitatively for levels of silver and copper. Dosing was based on gestation day 6 - 15 body weight.

2. Toxicological Evaluation of Dosed Animals:

Daily observations were made of all animals and any abnormal

conditions were recorded. Observations were made on the skin, eyes and mucous membranes, respiratory, circulatory, gastrointestinal and neuromuscular systems and behavior patterns. The death of any animal during the study period was recorded. Animals were weighed on gestation days 6, 7, 10, 13, 17 and 20 (Gestation day 6 was the day of randomization and the first day of dosing; gestation day 20 was the day of sacrifice). Food consumption was measured and recorded for a 24 hour period on days 10 and 17 of gestation.

After euthanization of all surviving animals (day 20 of gestation), necropsies were performed on the first 20 pregnant dams in groups 1, 2 and 4. The fetuses were removed by caesarean section and evaluated. In group 3, 18/30 (60%) dams were found to be pregnant, 10/30 (33%) not pregnant and 2/30 (7%) died prior to necropsy.

Gross necropsy/ Caesarian section:

The pregnant dams were macroscopically examined for any structural abnormalities or pathological changes that might have influenced the pregnancy. Gross examination was made of major organs including the heart, liver, lungs, spleen and kidneys. The uterus was removed and the contents were examined for embryonic or fetal deaths and the number of viable fetuses. The ovaries were removed and corpora lutea were counted using a dissecting microscope.

Fetal evaluation:

The fetuses were examined in the following manner: live and dead fetuses were counted and the time of death "in utero" was estimated, if possible. Resorptions were counted and defined as early or late. The sex of each fetus was determined. Each live fetus was weighed and the mean fetal weight was calculated for each litter. Half of the fetuses from each litter were preserved in 10% formalin and examined for visceral or soft tissue abnormalities. The other half of the fetuses from each litter were preserved in 70% ethanol, cleared and stained with Alizarin Red S for skeletal evaluation. The crown-rump length of each fetus was measured and the mean crown-rump length was calculated for each litter.

Historical control data were not provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed: Number Cruncher Statistical System was used to evaluate maternal and fetal data. All statistical tests were conducted at a 5% two-sided risk level.

Bartlett's test for homogeneity of variance was performed on:

- maternal body weight gain
- food consumption/100 g body weight
- fetal body weight
- crown-rump length

A one-way analysis of variance was used if the Bartlett's test was not significant. Duncan's multiple comparison test was performed to identify specific differences between the control and test groups when statistically significant differences were found at $p < 0.05$. If significant differences were observed in Bartlett's test, comparison with the control group was performed by the Wilcoxon's matched-pairs test.

Chi-square tests were performed on the number of live and dead fetuses, fetal sex ratios, and on fetal skeletal data from the vehicle control and test groups.

Compliance

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

C. RESULTS:

Mortality:

Of the 4 animals dying during the dosing period (day 6-15), 3/4 deaths were due to accidental puncture of the lung during dosing. On day 17 of gestation, one animal in the high dose group was hemorrhaging from the urogenital area. On necropsy, the kidneys were dark red and the stomach was distended with gas and silver copper zeolite. The caesarean section revealed 12 undeveloped fetuses, 1 late resorption and 17 corpora lutea on the ovaries. It would appear that the death of this rat was treatment-related.

Clinical Observations:

There were no abnormal signs noted in the control group. Wheezing was observed in 2/30 (6%), 6/30 (20%) and 8/30 (26%) rats in the low, mid and high dose groups, respectively. In the high dose group, sedation (37%), wheezing (26%), watery feces and urogenital discharge (10%) and thinness (6%) were observed. These clinical signs of toxicity appear to be related to treatment with silver copper zeolite.

Body Weight:

The maternal body weights for the first batch of mated females (control and mid-dose groups) were slightly higher, but not significantly, than for the second batch (low and high-dose groups). The two batches were received in the facility one week apart.

There was a statistically ($p < 0.05$) significant reduction in maternal body weight gain of those animals in the 2000 mg/kg dose group (Table 2 appended from study) in relation to controls. On days 10, 13, 17 and 20, there were decreased weight gains of 42%, 41%, 44% and 24%, respectively. There appears to be a treatment-related decrease in body weight gain during the dosing period (animals were dosed from day 6 - 15 of gestation) and possible recovery on day 17. According to the Author, Bartlett's test for homogeneity of variance was significant only at day 17 and Wilcoxon's matched-pairs test performed on day 17 indicated a significant difference between control and the high dose group. In addition, one-way ANOVA at each of the other time points, followed by Duncan's multiple comparison test when differences were found at $p < 0.05$, indicated that the high dose group had a significantly lower weight gain than the control at days 10, 13 and 20.

Food Consumption:

On day 17, there was a significant ($p < 0.05$) increase in food consumption in the low dose group (9.4 g/100 g body weight) over the control group (8.5 g/100 g body weight). One-way ANOVA followed by Duncan's multiple comparison test indicated a significantly ($p < 0.05$) lower amount of food consumed at the medium dose (8.2 g/100 g body weight) than the control group (8.8 g/100 g body weight) at day 10. There are no indications that these differences are related to administration of silver copper zeolite.

Gross Pathological Observations:

One female rat at the 200 mg/kg dose level exhibited an enlarged and irregularly shaped spleen. At the 2000 mg/kg dose level, one animal had an enlarged spleen and 2 had pale kidneys and livers. These effects are possibly due to silver copper zeolite.

The caesarean section data showed no significant differences between litters from control and treated animals for the numbers of live and dead fetuses (Table 6 & 7 appended from study). The mean number of early resorptions/litter for control, low-, mid- and high-dose groups was 0.1, 0.3, 0.2 and 0.4, respectively. In addition, there were no significant differences between litters from control and treated animals in terms of the mean litter

weight, mean fetal sex ratio and mean crown-rump length.

Fetal Evaluation:

Fetuses from all groups were examined externally for gross abnormalities. In the low dose group, 1 fetus had a hole in the palate. In the medium dose group, 1 fetus had its lower jaw hanging down and 2 incisors protruding. These findings were considered to be incidental and not related to dosing of the pregnant dams.

Examination of visceral and soft tissue of approximately one-half (434) of the fetuses yielded only one fetus with visceral abnormalities. A female in the 700 mg/kg (medium dose) group had an enlarged left kidney. This was considered to be an incidental finding.

The rest of the fetuses were examined for skeletal abnormalities which were categorized according to the presence of delayed ossification and/or abnormal bones. Evidence of litters with fetuses exhibiting delayed ossification, i.e., missing sternbrae and xiphisternum, absent or single vertebral centra, and absent vertebral arches, was seen at a rate of 79% for the vehicle control group, 75% for the low dose group, 83% for the medium dose group and 80% for the high dose group. The percent of total fetuses exhibiting delayed ossification was calculated to be 37, 38, 29 and 41% for the vehicle control, low, medium and high dose groups, respectively. There was no statistically significant difference between any of the groups when compared to controls. (Control values appear high. It would be helpful to have historical control data at this point.)

In the high dose group, 1/20 litters contained fetuses that exhibited significant skeletal abnormalities. One male fetus had misshapen radii, ulnae, femurs and wavy ribs. Two other males had wavy ribs. These abnormal fetuses were from a high dose dam that exhibited significantly decreased weight gain and decreased food consumption relative to controls. According to the Author, these effects were considered to be a consequence of evident maternal toxicity, and not indicative of a teratogenic effect.

D. DISCUSSION/CONCLUSIONS:

a. Maternal Toxicity:

There was no mortality among the dams as a result of the administration of silver copper zeolite. The daily observations showed an increased incidence of wheezing among the low-, mid- and high-dose animals at a rate of 6, 20 and 26%, respectively. Sedation, watery feces, urogenital discharge and thinness were also observed in the high dose group. Furthermore, a

statistically ($p < 0.05$) significant decrease in maternal body weight gain in the high dose group was observed.

b. Developmental Toxicity:

i. Deaths/Resorptions:

The mean number of uterine implantations, viable fetuses and resorptions were similar among control and treated groups.

ii. Altered Growth:

No treatment-related effects were noted.

iii. Developmental Variations/Malformations:

There were a few sporadic malformations noted in the visceral and skeletal examinations. One dam in the medium dose group had enlarged kidneys. This appears to be an incidental occurrence. The percentage of skeletal abnormalities by litter were similar among control and treated groups.

In the high-dose group, one litter (out of 20) contained fetuses that exhibited significant skeletal abnormalities including misshapen radii, ulnae, femurs and wavy ribs. Because these abnormal fetuses were from a dam that exhibited signs of maternal toxicity, the Author considers the skeletal abnormalities not indicative of a teratogenic effect.

D. Study Deficiencies:

Historical control data was not included.

E. Core Classification: Core - Supplementary Data.

This study satisfies most of the guideline requirements (83-3) for a developmental toxicity study. It can be upgraded with the submission of historical control data.

Maternal NOEL = 700 mg/kg

Maternal LOEL = 2000 mg/kg based on reduced body weight gain.

Developmental Toxicity NOEL = 700 mg/kg

Developmental Toxicity LOEL = 2000 mg/kg based on abnormal fetuses that exhibited signs of maternal toxicity.

Silver Zeolite DER

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