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p-DICHLOROBENZENE
GENETIC TOX BATTERY
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Summary Report

The Effect of Para-dichlorobenzene in a Battery of Genetic Toxicology Assays

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1. Introduction

The Interagency Testing Committee (ITC) recommended that para-dichlorobenzene be tested for a variety of health effects, including mutagenicity (Federal Register Vol. 15, No. 140, July 18, 1980). This summary report describes the results of the following tests:

Drosophila sex-linked recessive lethal, performed at the University of Wisconsin

In vitro CHO/HGPRT mutagenesis assay, performed at Bioassay Systems Corporation

In vitro chromosomal aberration assay in CHO cells, performed at Bioassay Systems Corporation

In vitro sister chromatid exchange assay in CHO cells, performed at Bioassay Systems Corporation

In vivo chromosomal aberration assay in rats, performed at Bioassay Systems Corporation

2. Chemical Identity

Para-dichlorobenzene is a solid at room temperature with a density of 1.2475 gm/cc at 20°C. The compound was supplied by Standard Chlorine Chemical Co. and had a purity of 99.7%. The lot number was 05181-P.

3. Results

3.1 *Drosophila* sex-linked recessive lethal assay

Wild-type males were exposed to para-dichlorobenzene by injecting a saturated air solution into a sealed vial. The concentration of para-dichlorobenzene in the exposure vial was calculated based on the percentage of air transferred into the vial.

In the first experiment, males were exposed to 1000 ppm for 6 hours; the dose was semi-narcotic indicating effective exposure to the males. The percent of lethals was 0.063 compared to the negative control value of 0.063. In the second and third experiments the males were exposed to approximately 3300 ppm for 4 hours; narcosis was observed followed by recovery. The percent of lethals was 0.039 and 0.024 in the two experiments compared to negative control data of 0.154 and 0.145.

Under conditions in which narcosis was observed, indicating that the males were exposed to the test compound, no increase in the frequency of sex-linked recessive lethals was observed due to para-dichlorobenzene.

3.2 In Vitro CHO/HGPRT Mutagenesis Assay

Para-dichlorobenzene was tested for toxicity and for induction of mutations at the HGPRT locus in CHO cells. The compound was tested under three conditions: nonactivated[1] (+serum), nonactivated (-serum) and activated (-serum). Under all three conditions, very little toxicity was observed until the cells were exposed to 200 ug/ml, which is above the solubility of para-dichlorobenzene in water (66 ug/ml, measured during this study, or 79 ug/ml, reported in the literature). The presence of serum in the exposure medium reduced the toxic effects of the compound. Based on these results, the compound was tested for mutagenicity under these three conditions, with concentrations ranging from 25 to 250 ug/ml.

In the nonactivated assays using serum, no increase in mutations was observed. The highest concentration tested, 250 ug/ml, resulted in less than 1% survivors.

Using nonactivated conditions without serum for a 4 hour exposure time, no increase in mutations was observed. Because of toxicity at higher concentrations data could only be obtained for exposures up to 200 ug/ml.

Using activated conditions, no increase in mutations was observed. Toxic effects were again observed at 250 ug/ml; and in one experiment no data could be obtained at this concentration.

Using the CHO/HGPRT system, under the three sets of conditions specified, para-dichlorobenzene did not induce an increase in mutations.

[1]Activation refers to the use of a rat liver S9 fraction to provide metabolic activation.

3.3 *In Vitro* Chromosomal Aberration Assay in CHO Cells

The compound was tested under nonactivated conditions using concentrations ranging from 20 to 300 ug/ml. No compound-related toxicity was seen at concentrations less than 300 ug/ml. Few metaphase cells were available for analysis at 300 ug/ml. There was no indication of an increased frequency of simple chromosome breaks or complex aberrations.

The compound was tested under activated conditions using concentrations from 20 to 300 ug/ml. Little toxicity was seen except 300 ug/ml where a reduction in the mitotic index was observed. There was no indication of an increase in the break frequency due to the compound.

3.4 *In Vitro* Sister Chromatid Exchange Assay in CHO Cells

The compound was tested in the nonactivated assay using concentrations ranging from 20 to 200 ug/ml. Since a low percentage of cells progressed through two cell cycles, no data was obtained at 200 ug/ml. No increase was seen in SCEs/cell at lower concentrations.

Under activated conditions, the compound was tested at concentrations ranging from 40 to 240 ug/ml. All cells were dead at 200 and 240 ug/ml, and there was no evidence for an increase in SCEs at lower concentrations of para-dichlorobenzene.

3.5 *In Vivo* Chromosomal Aberration Assay in Rats

Bone marrow cells from both male and female Sprague-Dawley rats exposed to para-dichlorobenzene by intra-peritoneal injection were analyzed for chromosomal damage. The doses tested were 200, 400 and 800 mg/kg since doses of 1000 mg/kg and higher were lethal. Animals were sacrificed at 6, 12 and 24 hours after dosing for preparation of bone marrow cells for analysis. Average negative control break frequencies for the three time points for the male and female rats were 0.5% and 0.6% respectively. These are similar to the values obtained in other experiments conducted at this testing laboratory. The average break frequencies of animals exposed to para-dichlorobenzene ranged from 0.0% to 1.2%. There was no statistically significant increase in the frequency of chromosomal damage in the test animals.

3.6 Conclusion

Under the conditions of the assays para-dichlorobenzene did not exhibit a detectable mutagenic response in either *Drosophila* or in cultured CHO cells. The compound did not induce any SCEs in CHO cells or any chromosomal aberrations in either CHO cells or in rats. For the three cell culture assays and the *in vivo* cytogenetic study, chemical analysis verified the stock concentrations used in the experiments. Chemical analysis was not performed during the Drosophila test, but exposure conditions were calculated using the physical characteristics of para-dichlorobenzene. For all assays the compound was tested to the limits of toxicity.