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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 27 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 2,4-DP: Review of an in vitro cytogenetic assay measuring chromosomal aberrations in Chinese hamster ovary cells (Addendum to Toxicology Chapter of 2,4-DP Registration Standard)

TO: Richard Mountfort / C. Rice, PM (23)
Registration Division (TS-767c)

FROM: Whang Phang, Ph.D.
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THROUGH: Marcia van Gemert, Ph.D. 7/25/88
Section Head
and
William Burnam, Deputy Branch Chief
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The registrant, Rhone Poulenc, submitted a report on an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary cells. The study has been reviewed, and the conclusion is that with metabolic activation 2,4-DP at concentrations of 2000 ug/ml or above induced chromosomal aberrations. In the abscence of the metabolic activation, the test compound was considered to be negative. The study is classified as Acceptable.

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Reviewed by: Whang Phang, Ph.D. 7/15/88
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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro cytogenetic assay measuring chromosomal aberration frequencies in CHO cells

EPA ACCESSION NO.: 405819-01

CASWELL NO.: 320

EPA RECORD NO.: 220389

EPA PROJECT NO.: 8-0845

SPONSOR: Rhone-Poulenc AG Co.

CAS No.: 120-36-50

TESTING LABORATORY: Hazleton Laboratories America, Inc.
Kensington, Maryland

CITATION: Murli, H. (1988). Mutagenicity test on 2,4-DP Tech in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. Hazleton Laboratories America Inc.; Study No.: 10158-0-437; March 21, 1988. Submitted by Rhone-Poulenc AG Co., April 8, 1988.

SUMMARY: In in vitro cytogenetic assays measuring chromosomal aberration frequencies in CHO cells, with metabolic activation 2,4-DP at concentrations of 2000 ug/ml or above induced chromosomal aberrations. In the absence of the metabolic activation, the test compound was considered to be negative.

METHODS AND MATERIALS:

Test Compound: 2,4-DP Technical 96.83% pure;
off-white powder; Lot # 9-L3P-94

Cells: Chinese hamster ovary cells (CHO-WBL) were obtained from a permanent cell line from the laboratory of Dr. S. Wolff, University of California, San Francisco. The cells have been recloned to maintain karyotypic stability.

Postive control agents: Mitomycin C (MMC) was used in assays without metabolic activation.

Cyclophosphamide (CP) was used in assays with metabolic activation.

EXPERIMENTAL PROCEDURES:

The details of the experimental procedures are excerpted from the submitted study (HLA Study No.: 10158-0-437) and presented in Appendix 1. Briefly, dose-range finding assays were conducted with and without metabolic activation for assessing delay of cell cycle progression. Based upon the results of dose-range finding study, a delayed 20 hour fixation was selected, and

duplicate cultures of CHO cells were incubated with 75.1 through 1000 ug/ml in the nonactivation assays and 1000 through 3000 ug/ml in assays with metabolic activation, which consisted of rat liver S9 fraction. In the positive control assays, MMC at concentrations of 0.04 and 0.08 ug/ml and CP at concentrations of 12.5 and 17.5 ug/ml were used, but only cells from one dose were actually analyzed for each set of aberration assays. For negative and solvent controls only single cultures were used. Chromosomal aberrations were analyzed from one dose of the positive control doses and from four highest doses of test agent treated cultures. The definitions for chromosomal aberrations are presented in Appendix 2.

RESULTS:

A. Range-finding Study:

Dimethyl sulfoxide (DMSO) was found to be the solvent of choice. A clear amber solution was obtained in DMSO at a concentration of 494 mg 2,4-DP/ml DMSO whereas, in the culture medium, 100 mg 2,4-DP/ml culture medium produced a heavy and thick suspension. Hence, a stock solution of 301 mg/ml was prepared with DMSO, and from this solution, a half log series of concentrations of 0.1 through 3010 ug/ml was tested in the range-finding study.

In the studies without metabolic activation, at 3010 ug/ml there was complete cellular toxicity. At 1000 ug/ml, no apparent effect on the cellular monolayer confluence was seen, but there were cellular debris and decreased mitotic cells. Analyses of cell cycle kinetics indicated significant dose related cell cycle delay at concentrations of 100, 301, and 1000 ug/ml (Table 1A). Therefore, for the aberration assays without metabolic activation, a series of concentrations of 75.1, 100, 501, 751, and 1000 ug/ml was selected.

For the studies with metabolic activation, at 3010 ug/ml, a visible precipitate, floating cellular debris, few visible mitotic cells, and a 25% reduction in cellular monolayer were seen (Table 1B). No obvious toxicity was observed at doses lower than 3010 ug/ml. Cell cycle delays were also found at 1000 and 3010. Doses of 1000, 1500, 2000, 2500, and 3000 ug/ml were selected.

A fixation time of 20 hours was chosen for all the aberration assays.

B. Chromosomal aberration assay without metabolic activation:

At 751 ug/ml, there was a small, but statistically significant increase in the percent of cells with aberrations. However, here were no dose-related response, and no significant com-

plex rearrangements like triradials or quadriradials (Tables 2 & 3). The test article was considered negative for inducing chromosomal aberrations in the absence of metabolic activation.

C. Chromosomal aberration assay with metabolic activation:

There were significant increases in percent of cells with chromosomal aberrations at concentrations of 2000 ug/ml or above (Tables 4 & 5), and these aberrations included both simple and complex rearrangements. It should be noted that at concentrations of 2000 ug/ml or above precipitate and floating cellular debris were observed. At 1500 ug/ml, there was an initial precipitate, but the test agent soon went into solution. In addition, cytotoxicity and increased chromosomal aberrations were not seen 1500 ug/ml. Based on these results, 2,4-DP acid was considered positive for inducing chromosomal aberrations in the presence of metabolic activation.

DISCUSSION AND CONCLUSION:

Under the conditions of metabolic activation, 2,4-DP at concentrations of 2000 ug/ml or above induced marked chromosomal aberrations, which consisted of both simple and complex rearrangements in CHO cells. In the absence of metabolic activation, however, the compound was considered negative for inducing chromosomal aberrations at tested concentrations of 1000 ug/ml or less. The study is classified as Acceptable.

2,4-DP revised

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