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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 2,4-DP BUTOXYETHYL ESTER: Review of in vitro cytogenetics using Chinese hamster ovary cells.

Caswell No.: 320E EPA Accession NO.: 404317-01

TO: Richard Mountfort, PM (23)
Registration Division (767c)

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THROUGH: Marcia van Gemert, Ph.D. 5/3/88
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The registrant Rhone-Poulenc has submitted a mutagenicity study - chromosomal aberration assays using Chinese hamster cells. The study has been evaluated, and Data Evaluation Record is attached. The conclusion is as follows:

> Cultured Chinese hamster ovary cells were treated with 2,4-DP butoxyethyl ester at concentrations ranging from 100 to 851 ug/ml without metabolic activation and from 100 to 1000 ug/ml with metabolic activation. Increased incidences of chromosome and chromatid breaks were observed in cells treated with 751 and 851 ug/ml in the absence of the metabolic activation. The data suggested that 2,4-DP butoxyethyl ester was a direct acting clastogen under these experimental conditions.

This review is an addendum to the 2,4-DP Registration Standard which has been completed by Toxicology Branch since January, 1988.

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity: in vitro cytogenetics using Chinese hamster ovary cells

EPA ACCESSION No.: 404317-01 TOX. No.: 320E

EPA RECORD No.: 212971 PROJECTION No.: 8-0578

CHEMICAL: 2,4-DP Butoxyethyl ester (Technical)

SPONSOR: Rhone-Poulenc Ag Co., Research Triangle Park, NC

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

CITATION: Murli, H. (1987). 2,4-DP butoxyethyl ester in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. HLA Study No.: 985-0-437, Hazleton Laboratories America, Inc.; Aug 26, 1987. Submitted by Rhone Poulenc (12/4/87).

CONCLUSION:

Cultured Chinese hamster ovary cells were treated with 2,4-DP butoxyethyl ester at concentrations ranging from 100 to 851 ug/ml without metabolic activation and from 100 to 1000 ug/ml with metabolic activation. Increased incidences of chromosome and chromatid breaks were observed in cells treated with 751 and 851 ug/ml in the absence of the metabolic activation. The data suggested that 2,4-DP butoxyethyl ester was a direct acting clastogen under these experimental conditions.

This study is classified as Acceptable.

Methods and Materials: The details of the experimental procedures are presented in the Appendix.

Test material: 2,4-DP Butoxyethyl ester

The report did not specify the purity of the test chemical, and no chemical analysis was reported. However, the Registration Division Data Review Record stated that this study was submitted under 264-231, a 59% end-use product. More information was requested by this reviewer. The registrant informed the product manager by telephone that the test chemical was technical grade. Later a written statement indicated that the test article was 100% technical.

For dose selection, the solubility of the test agent was tested in the culture medium.

Chinese hamster ovary (CHO) cells: CHO cells were originally obtained from the University of California, San Francisco. The cells were from a stable cell line and were grown in McCoy's 5a culture medium which was supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, and 1% penicillin and streptomycin.

Metabolic activation: S9 reaction mixture was derived from the liver of male Sprague-Dawley rats which were treated with Aroclor to induce mixed function oxidase.

Controls: Positive controls used in this study were mitomycin C (MMC)(0.25 and 0.5 ug/ml for range finding study; 0.04 and 0.08 ug/ml for chromosomal aberration assays) for non-activation assays and cyclophosphamide (CP)(20 and 25 ug/mg for range finding study; 25 and 50 ug/ml for chromosomal aberration assays) in the metabolic activation series.

Range-finding: For the non-activation assay, approximately 0.3 x $10^6$ CHO cells per 25 $cm^2$ flask in 5 ml of McCoy's 5a culture medium were exposed to the test agent at doses ranging from 0.0336 ug/ml to 1010 ug/ml in half log series. The report did not clearly state the length of time the cells were treated with the test agent. However, it was reported that 5-bromo-2'-deoxyuridine (BrdUrd) was added at a final concentration of 10 uM two hours after initial exposure to the test agent and then incubated for approximately 23 hours. The treated cells were washed 2.5 hours prior to the harvest of cells, and at that time Colcemid® (10 ug/ml) was added. The harvested cells were stained and analyzed for cell cycle delay. For metabolic activation assays, CHO cells were exposed to the test agent and the S9 reaction mixture for 2 hours at 37°C. After the exposure period, the cells were washed, incubated with BrdUrd for 23 hours, and treated with Colcemid for 2.5 hours. Finally the cells were harvested, stained, and analyzed.

Chromosomal Aberration Assays: Approximately 1.2 to 1.5 x $10^6$ CHO cells were seeded in a 75 $cm^3$ flask. For non-metabolic activation assays, one day following culture initiation the cells were treated with 2,4-DP butoxyethyl ester at doses of 250, 376, 501 and 751 ug/ml for 17.3 hours. The cells were then washed with buffered saline; 0.1 ug/ml colcemid® in McCoy's 5a medium was added to the cells and incubated for an additional 2.5 hours. Subsequently, the cells were

harvested, fixed, placed on slides, stained and analyzed for chromosomal aberrations. For metabolic activation assays, the cells were treated with the test agent in the presence of S9 reaction mixture for 2 hours. The cells were washed with buffered saline and incubated in McCoy's culture 5a medium for an additional 7.8 hours with 0.1 ug/ ml Colcemid® for the last 2.5 hours of incubation. The metaphase cells were then harvested and prepared for cytogenetic analysis.

One hundred cells from each duplicate culture treated with test agent were analyzed. For positive controls only 25 cells were scored for chromosomal aberrations.

## RESULTS

The limit of solubility of 2,4-DP butoxyethyl ester was found to be 1.0 mg/ml. At concentrations higher than 1.0 mg/ml, the test agent formed globules, precipitated out of the solution, and adhered to the plastic tube. DMSO was found to be the solvent of the choice for preparing the stock solution (100 mg/ml) from which other dosing concentrations were prepared. A concentration range of 0.0333 through 1000 ug/ml was selected for the range-finding studies.

Range-finding study: The results of dose-range finding studies are presented in Table 1. For the study without metabolic activaction, the concentrations of test agent were 33.6, 101, 336, and 1010 ug/ml. At 1010 ug/ml, toxicity of the cells was observed as indicated by complete absence of monolayer. At 336 ug/ml, a 25% reduction of monolayer confluence with few observable mitotic cells relative to the controls was observed. Cells treated with the two lower concentrations did not show any difference from that of the controls. Cell cycle kinetics were also examined at concentrations of 33.6, 101, and 336 ug/ml; there were marked differences in cell cycle delay at all concentrations relative to the control. For chromosomal aberration assays without metabolic activation, the concentrations of 100, 175, 250, 375, 500, and 750 ug/ml and an incubation period of 20 hours with the test agent were selected.

For dose-range-finding assays with metabolic activation, the concentrations of test agent were 101, 336, and 1010 ug/ml. At 1010 ug/ml, reduced monolayer confluence, with an unhealthy cell monolayer, and reduced visible mitotic cells were observed. The data on the cell cycle kinetics indicated that 1010 ug/ml did not sufficiently delay the cell cycle to

to alter the culture time. Based upon these results, a 10 hour treatment period with a total incubation time of 10 hours and concentrations of 100, 250, 500, 750, and 1000 ug/ml were selected for the final chromosomal aberration assays.

Chromosomal aberration assays:

Without activation: The results are summarized in Table 2. Marked increases in the incidence of chromosome and chromatid breaks were observed at 751 ug/ml relative to the controls. Slight increases in the incidences of triradials in 76, 502, and 751 ug/ml and of quadriradials in 751 ug/ml were seen. These data appeared to indicate that 2,4-DP butoxyethyl ester was a direct acting clastogen.

An additional experiment was carried out using closely related doses (651, 751, and 851 ug/ml) to further study the effects of the test agent. The summary results are presented in Table 3. An increased incidence of chromatid breaks was seen in cells treated with 651, 751, and 851 ug/ml. There was a significant increase in percent of cells with aberrations at 851 ug/ml.

With activation: The results of chromosomal aberration assays in the presence of metabolic activation are presented in Table 4. The chromosomal aberration analyses were performed on cells treated with 250, 501, 751, and 1000 ug/ml of the test agent. The result did not indicate any notable increase in the incidence of chromosomal aberrations in the treated cells relative to the controls.

DISCISSION

Based upon the reported results, 2,4-DP butoxyethyl ester at 751 ug/ml or above appeared to be a direct acting clastogen causing an increased incidence of chromosome and chromatid breaks.