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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

MAY 23 1996

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CASWELL FILE

011937

MEMORANDUM

SUBJECT BUTOXYETHYLHEXYL ESTER OF 2,4-DICHLOROPHENOXYACETIC ACID: Review of a CHO/HGPRT Forward Mutation Assay.

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

FROM: Jess Rowland, M.S., Toxicologist Section I, Toxicology Branch II, Health Effects Division (7509C)

TO: Walter Waldrop / Judy Coombs Product Manager 71 Reregistration Division (7508W)

THRU: Yiannakis Ioannou, Ph.D Head Section I, Toxicology Branch II, Health Effects Division (7509C)

and Stephanie Irene, Ph.D., Acting Chief Toxicology Branch II, Health Effects Division (7509C)

DATA PACKAGE IDENTIFICATIONS: Submission: S475220 DP Barcode: D208352 PC Code: 030053 Caswell No. 315A1

ACTION REQUESTED: Review of CHOR/HGPRT forward mutation study (MRID #43394201) to satisfy Guideline requirement §84-2(2).

RESPONSE: A Data Evaluation Record for the report titled "Evaluation of 2,4-dichlorophenoxyacetic acid butoxyethyl ester in the Chinese Hamster Ovary Cell/Hypoxanthine-guanine-phosphoribosyl Transferase (CHO/HGPRT) forward mutation assay" (MRID 43394201) is attached. The Executive Summary is as follows:

EXECUTIVE SUMMARY: In an in vitro mammalian cell gene forward mutation assay, CHO/HGPRT, (MRID 43394201) Chinese hamster ovary (CHO) cells cultured in vitro were exposed to 2,4-D butoxyethyl ester (94.6% a.i.) at concentrations of 600, 700, 800, 900, or 1,000 µg/mL in an initial assay and 200, 300, 400, 500, 600, or 700 µg/mL in two subsequent assays in the absence of S9 activation. In an initial and confirmatory assay, with an S9 activated system, cells were exposed to five levels ranging from 150-1,400 µg/mL. The S9 fraction was derived from Aroclor-induced rat liver. The test material was delivered to the test system in dimethylsulfoxide (1.0%, v/v). 2,4-D BEE was very cytotoxic in the non-activated CHO cell system at dose levels at or above 800 µg/mL. The test material was relatively non-cytotoxic in the S9-activated system at any dose level. There was no evidence of mutagenic effect at any dose level with or without activation. Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis.

This study is classified as acceptable, and satisfies the requirements for the 1991 Guideline §84-2(2) for an in vitro mutagenicity (mammalian forward gene mutation) data.



DATA EVALUATION REPORT**BUTOXYETHYL ESTER OF 2,4-D**

Study Type: 84-2; Mammalian Cells in Culture; Gene Mutation Assay in
Chinese Hamster Ovary Cells (CHO/HGPRT)

Dynamac Study No. 111A (MRID 43394201)

Prepared for

Health Effects Division
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Prepared by

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Primary Reviewer:

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Date: 1/16/96

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Stephen Brecher, Ph.D

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Date: 1/16/96

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William J. Spangler, Ph.D.

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Date: 1/16/96

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Reto Engler, Ph.D.

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Date: 1/16/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

[2,4-D BEE]

MAMMALIAN CELLS IN CULTURE: GENE MUTATION (84-2)

EPA Reviewer: Jess Rowland, M.S, Toxicologist *JR* Date 5/15/96
 Review Section II, Toxicology Branch II(7509C)

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D., Head *YI* Date 5/16/96
 Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay (CHO/HGPRT)

DP BARCODE: D208352

SUBMISSION: S475220

P.C. CODE: 030053

TOX. CHEM. NO.: 315AI

TEST MATERIAL.: 2,4-Dichlorophenoxyacetic acid butoxyethyl ester

SYNONYMS: 2,4-D BEE

CITATION: Linscombe, V., and S. Lick (1994) Evaluation of 2,4-dichlorophenoxyacetic acid butoxyethyl ester in the Chinese Hamster Ovary Cell/Hypoxanthine-guanine-phosphoribosyl Transferase (CHO/HGPRT) forward mutation assay. The Toxicology Research Laboratory, Midland, MI. Laboratory Project Study ID: K-007722-023. October 3, 1994. MRID 43394201.

SPONSOR: DowElanco, Indianapolis, IN

EXECUTIVE SUMMARY: In an *in vitro* mammalian cell gene forward mutation assay, CHO/HGPRT, (MRID 43394201) Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to 2,4-D butoxyethyl ester (94.6% a.i.) at concentrations of 600, 700, 800, 900, or 1,000 $\mu\text{g}/\text{mL}$ in an initial assay and 200, 300, 400, 500, 600, or 700 $\mu\text{g}/\text{mL}$ in two subsequent assays in the absence of S9 activation. In an initial and confirmatory assay, with an S9 activated system, cells were exposed to five levels ranging from 150-1,400 $\mu\text{g}/\text{mL}$. The S9 fraction was derived from Aroclor-induced rat liver. The test material was delivered to the test system in dimethylsulfoxide (1.0%, v/v).

2,4-D BEE was very cytotoxic in the non-activated CHO cell system at dose levels at or above 800 $\mu\text{g}/\text{mL}$. The test material was relatively non-cytotoxic in the S9-activated system at any dose level. **There was no evidence of mutagenic effect at any dose level with or without activation.** Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis.

This study is classified as acceptable, and satisfies the requirements for the 1991 Guideline §84-2(2) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 2,4-Dichlorophenoxyacetic acid butoxyethyl ester

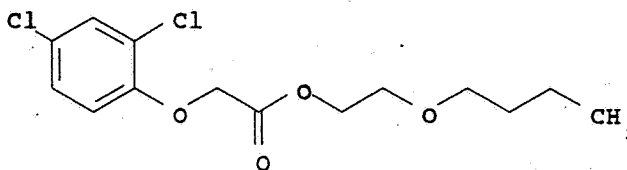
Description: Amber liquid

Lot/Batch #: AGR 276426

Purity: 94.6% a.i.

Stability of compound: Not reported

CAS #: 1929-73-3



Solvent used: Dimethylsulfoxide (DMSO)

Other comments: None

2. Control Materials:

Solvent/final concentration: 1% DMSO in culture medium

Positive: Non-activation (concentrations, solvent):

Ethylmethanesulfonate (EMS)/621 µg/mL in culture medium

Activation (concentrations, solvent):

20-Methylcholanthrene (20-MCA)/4 µg/mL in DMSO/culture medium

3. Activation: S9 derived from

- | | | | |
|--|---|---|---|
| <input checked="" type="checkbox"/> Aroclor 1254 | <input checked="" type="checkbox"/> induced | <input checked="" type="checkbox"/> rat | <input checked="" type="checkbox"/> liver |
| <input type="checkbox"/> phenobarbital | <input type="checkbox"/> non-induced | <input type="checkbox"/> mouse | <input type="checkbox"/> lung |
| <input type="checkbox"/> none | | <input type="checkbox"/> | <input type="checkbox"/> hamster |
| <input type="checkbox"/> other | | <input type="checkbox"/> | <input type="checkbox"/> other |

S9 was purchased from Sitek Research Laboratories, Rockville, MD. S9 mix composition: S9 (10% v/v), 10 mM MgCl₂ · 6 H₂O, 5 mM glucose-6-phosphate, 4 mM NADP, 10 mM CaCl₂, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0)

4. Test Cells: Chinese hamster ovary (CHO) cells

Properly maintained? **Yes**

Periodically checked for Mycoplasma contamination? **Yes**

Periodically checked for karyotype stability? **Not reported**

Periodically "cleansed" against high spontaneous background? **Not reported**

Media: Ham's F-12 nutrient mix supplemented with 5% heat-inactivated fetal calf serum; 25 mM HEPES; Fungizone, penicillin G; and streptomycin sulfate.

5. Locus Examined: thymidine kinase (TK)

Selection agent: _____ bromodeoxyuridine (BrdU)

_____ fluorodeoxyuridine (FdU)

_____ trifluorothymidine (TFT)

x hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)

Selection agent: _____ 8-azaguanine (8-AG)

10 µM 6-thioguanine (6-TG)

 Na⁺/K⁺ ATPase

Selection agent: _____ ouabain

(give concentration)

 other (locus and/or selection agent; give details):

6. Test compound concentrations used:

Non-activated conditions:

Cytotoxicity assay: 150, 300, 600, 800, 1,000, 1,200, 1,400 µg/mL

Gene mutation Assay 1: 600, 700, 800, 900, 1,000 µg/mL

Gene mutation Assay 2: 200, 300, 400, 500, 600, 700 µg/mL

Gene mutation Assay 3: 200, 300, 400, 500, 600, 700 µg/mL

Activated conditions:

Cytotoxicity assay: 150, 300, 600, 800, 1,000, 1,200, 1,400 µg/mL

Gene mutation Assay 1: 150, 300, 600, 1,000, 1,400 µg/mL

Gene mutation Assay 2: 200, 300, 600, 1,000, 1,400 µg/mL

B. TEST PERFORMANCE**1. Cell treatment:**

a. Cells exposed to test compound, negative/solvent or positive controls for: 4 hours (non-activated) 4 hours (activated)

b. After washing, cells cultured for 6-8 days (expression period) before cell selection

c. After expression, 2×10^5 cells/dish (2 dishes/ group) were cultured for 7-9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7-9 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 cells was evaluated using weighted analysis of variance. Treated groups were compared to the vehicle control using a linear trend test and lack of fit test ($\alpha=0.05$). In the event of a significantly increasing trend or significant lack of fit, a Dunnett's t-test was conducted, and additional comparison of the positive control to the negative control was conducted using a linear contrast statement.

3. Evaluation Criteria: An assay was considered acceptable if the mutation frequency in the positive controls were significantly higher than the negative controls and if the negative controls were within reasonable limits of the laboratory historical control and literature values.

The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutation frequency compared to the vehicle control.

II. REPORTED RESULTS

- A. **Analytical determinations:** Analyses (HPLC) of the test material stock solutions from 300-1,400 $\mu\text{g}/\text{mL}$ indicated that the actual concentrations were 102-104% of the target concentrations.
- B. **Preliminary cytotoxicity assay:** The results are presented in Attachment 1 (study report page 20). The cytotoxicity test was conducted with seven concentrations of 2,4-D BEE ranging from 150 to 1,400 $\mu\text{g}/\text{mL}$ with or without S9 activation. The highest concentration was based upon limitations posed by the solubility of the test substance; at 1,200 and 1,400 $\mu\text{g}/\text{mL}$ the test material precipitated following addition to the culture medium. In the non-activated cultures, excessive toxicity ($\leq 1\%$ relative cell survival, RCS) was observed at the 1,200- and 1,400- $\mu\text{g}/\text{mL}$ dose levels. RCS was 10.2% at 1,000 $\mu\text{g}/\text{mL}$. In the presence of S9, RCS was 51.8% at the highest dose level. Based on these results, dose levels of 600-1,000 $\mu\text{g}/\text{mL}$ were chosen for the test without S9 and dose levels of 150-1,400 $\mu\text{g}/\text{mL}$ were selected for the test with S9 activation.
- B. **Mutagenicity assay:** The results are presented in Attachment 2 (study report pages 21-25). The test chemical at concentration of 1,000 $\mu\text{g}/\text{mL}$ formed a precipitate upon addition to the culture medium. In non-activation assay 1 (Table 2A), cultures treated at ≥ 800 $\mu\text{g}/\text{mL}$ failed to proliferate during the expression period. As a result of the toxicity observed in Assay 1, the test concentrations for assays 2 and 3 were lowered to 200-700 $\mu\text{g}/\text{mL}$ (Tables 2B and 2C). In the presence of S9, the test article was minimally cytotoxic at the highest dose levels (Tables 3A and 3B). The mutation frequencies in cultures treated with the test material in the absence and presence of S9 were not significantly different from the concurrent negative controls and were within the laboratory historical background range of 0-2.6 (minimum) and 9.3-27.9 (maximum) TG⁺ mutants per 10^6 clonable cells over a 10-year period. In all assays, the positive control chemicals EMS (non-activated assay) and 20-MCA caused significant increases in mutation frequencies. Based on these results, the study authors concluded that 2,4-D BEE was not mutagenic in this *in vitro* mammalian cell (forward mutation) test system.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

- A. The reviewer agrees with the study authors, that 2,4-D BEE did not induce mutations in this CHO/HGPRT mammalian forward gene mutation assay when tested to a cytotoxic level and the limit of solubility. The sensitivity of the test system to detect a mutagenic response was clearly demonstrated by the significant results obtained with the positive control substances, 621 $\mu\text{g}/\text{mL}$ EMS in the non-activated system and 4 $\mu\text{g}/\text{mL}$ 20-MCA in the S9 activated system. The reviewer concludes that 2,4-D butoxyethyl ester was not mutagenic in this *in vitro* forward gene mutation system.
- B. **Study deficiencies:** None.

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ATTACHMENTS

TR Review 011937

Page _____ is not included in this copy.

Pages 9 through 13 are not included in this copy.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
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 - 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.
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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.
