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

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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

FROM: Jess Rowland, M.S, Toxicologist 5/16/96
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TO: Walter Waldrop / Judy Coombs
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THRU: Yiannakis Ioannou, Ph.D Head 5/17/96
Section I, Toxicology Branch II, Health Effects Division (7509C)
and
Stephanie Irene, Ph.D., Acting Chief 5/23/96
Toxicology Branch II, Health Effects Division (7509C)

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

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.

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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

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Office of Pesticide Programs
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Disclaimer

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

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

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D., Head 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 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.

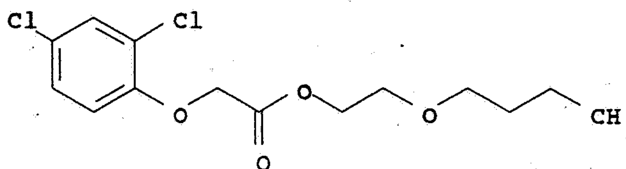
This study is classified as **acceptable**, and satisfies the requirements for the 1991 Guideline §84-2(2) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ hamster	__ other
__ other		__ other	

S9 was purchased from Sitek Research Laboratories, Rockville, MD. S9 mix composition: S9 (10% v/v), 10 mM $MgCl_2 \cdot 6\ H_2O$, 5 mM glucose-6-phosphate, 4 mM NADP, 10 mM $CaCl_2$, 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 µg/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 µg/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 µg/mL the test material precipitated following addition to the culture medium. In the non-activated cultures, excessive toxicity (≤1% relative cell survival, RCS) was observed at the 1,200- and 1,400-µg/mL dose levels. RCS was 10.2% at 1,000 µg/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 µg/mL were chosen for the test without S9 and dose levels of 150-1,400 µg/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 µg/mL formed a precipitate upon addition to the culture medium. In non-activation assay 1 (Table 2A), cultures treated at ≥800 µg/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 200-700µg/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^r 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 µg/mL EMS in the non-activated system and 4 µg/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

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______ Description of quality control procedures.

______ Identity of the source of product ingredients.

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______ A draft product label.

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