5-29-91

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

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MAY 29 1991

MAY 29 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of 2,4-DP Mutagenicity Studies

TO: Lois Rossi PM 74
SRRD (H7508C)

FROM: Karen E. Whitby Ph.D. 5/13/91
Section, II
Toxicology Branch II/(HED) (H7509C)

THRU: K. Clark Swentzel 5/17/91
Section Head
Toxicology Branch II/(HED) (H7509C)

and

Marcia van Gemert, Ph.D. 5/23/91
Chief, Toxicology Branch II/(HED) (H7509C)

HED Project No. 1-0479
Caswell No. 320

Action Requested

Please review tox. data for 2,4-DP acid in response to Registration Standard, guideline reference nos. 84-2a, 84-2b, and 84-4. The studies were secondarily reviewed by Dr. John Chen of Tox. Branch II. The citation for the studies and the reviewer's conclusions are excerpted from the Data Evaluation Report and are presented below:

Data Evaluation Reports for three 2,4-DP mutagenicity studies are attached:

**1) MRID 416468-01 MUTAGENICITY TESTING - 2,4-Dichlorophenoxypropionic Acid in the Ames Test**

Five concentrations of 2,4-Dichlorophenoxypropionic acid (2,4-DP) ranging from 20 to 5000 µg/plate were evaluated in the Salmonella typhimurium mammalian microsome mutagenicity assay. The test material was cytotoxic in all strains at the two highest doses (2500 and 5000 µg/plate +/-S9) but failed to induce a mutagenic

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response in S. typhimurium TA1535, TA1537, TA1538, TA98, or TA100. However, the S9-activated assay was conducted with an excessive concentration of S9 (30%) in the S9 reaction mixture. Unless the author can justify the use of a high S9 concentration, the assay should be repeated using the recommended screening concentration (4% S9 in the S9 mix). Additionally, analytical data to support the actual concentrations of the test material used in the assay and a statement of quality assurance were not provided.

The study, therefore, does not fulfill the Guideline requirements for genetic effects, Category I, Gene Mutations.

Study Classification: The study is unacceptable.

**2) MRID 416468-02 MUTAGENICITY TESTING - Cytogenetic Investigations of 2,4-DP In Chinese Hamsters - Bone Marrow Chromosome Analysis**

The potential of 2,4-DP to induce chromosome damage was investigated in Chinese hamsters. Groups of 10 animals (5 males and 5 females) received single oral gavage administrations of 47, 280, or 1780 mg/kg of the test material and were sequentially sacrificed 6, 24, and 48 hours following treatment. Toxic signs, which included death in one female, apathy, atony, irregular respiration, squatting posture, and piloerection, were seen in the high dose group. Similar but less intense toxic effects were also observed in the mid dose group. These toxicological signs were consistent with those observed in a preliminary acute oral toxicity range-finding study conducted with the test material.

Although the data indicate that an appropriate range of test material doses was evaluated for this in vivo study, no definitive conclusions could be reached regarding the potential clastogenesis of 2,4-DP. Significant ($p \leq 0.01$) increases in the percentage of aberrant cells were observed in the 1780 mg/kg dose group at the 24 and 48 hour harvest interval. However, these increases did not appear to be time or dose dependent and were limited to the high dose group. Our ability to independently review the data was further limited for the following reasons:

1. Only aberrations classified as exchanges or cells with pulverized chromosomes or multiple aberrations (i.e., $\geq$ 5 aberrations/cell) were identified.

2. Classifying cells with $\geq$ 5 aberrations/cell as multiple aberrations does not conform with the generally accepted approach (i.e., multiple aberrations are cells with $\geq$ 10 aberrations/cell) and further limits the independent assessment of the data. The type and frequency of specific aberrations is as important a determinant of clastogenesis as is the frequency of cells with aberrations.

3. Mid and low dose groups from the 6 hour and 48 hour harvest were not scored; at minimum, the 48 harvest cells should have been analyzed. We do, however, concur that the mitotic indices for the high dose group at 6, 24, and 48 hours posttreatment did not suggest a delay in cell cycling.

4. Since dosing was based on animal weight data, individual bodyweights should have been included in the report.

However, the findings of an in vivo sister chromatid exchange (SCE) assay in Chinese hamsters administered comparable doses of the test material showed clear dose related genotoxic effects; the SCE frequencies were significantly ($p<0.01$) increased in the mid (280 mg/kg) and high dose (1780 mg/kg) groups (see Data Evaluation Record 362-C). Since SCE induction frequently occurs at doses lower than required to induce chromosomal aberrations, the SCE assay is considered to be a sensitive indicator of clastogenesis. Hence, the SCE assay results provide additional support that 2,4-DP may be a clastogen.

Since definitive conclusions cannot be reached, the study does not satisfy Guideline requirements for genetic effects Category II, Structural Aberrations. However, we classify 2,4-DP as presumptively positive.

Study Classification: The study is unacceptable; however, 2,4-DP is classified as presumptively positive.

Recommendation: The study should be repeated with consideration given to the study deficiencies outlined above.

**3) MRID 416468-03 MUTAGENICITY TESTING - Cytogenetic Investigations of 2,4-DP in Chinese Hamsters - Sister Chromatid Exchange**

The potential of 2,4-DP to induce sister chromatid exchanges (SCE) was investigated in Chinese hamsters. Groups of 10 animals (5 males and 5 females) received single oral gavage administrations of 47, 280, or 1780 mg/kg of the test material and were sacrificed 24 hours after treatment. Toxic signs which included death, apathy, atony, irregular respiration, squatting posture, and piloerection were seen in the high dose group. Similar but less toxic effects were also observed in the mid dose group. These toxicological signs were consistent with those observed in a preliminary acute oral toxicity range-finding study conducted with the test material.

The analysis of metaphases showed a clear dose-related increase in the frequency of SCEs; the findings for the mid and high dose groups were significant ($p<0.01$). We disagree with the study author's claim that the induced response was weak. The intensity of the response could not be determined because conditions were not optimized for SCE detection. The subcutaneous implantation of 5-bromodeoxyuridine (BrdU) pellets into hamster necks in conjunction

with compound administration 2 hours postimplantation may have lessened assay sensitivity and, therefore, limited the full expression of the genotoxic response. We, nevertheless, classify 2,4-DP as positive in this in vivo test system. Based on overall results, the study satisfies Guideline requirements for Category III, Other Mutagenic Mechanisms.

Study Classification: The study is acceptable; 2,4-DP is positive for the induction of SCEs in Chinese hamsters.

Recommendations: It is suggested that future in vivo SCE assays conducted by the performing laboratory follow the recommended approach for BrdU administration.

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EPA No.: 68D80056
DYNAMAC No.: 362-A
TASK No.: 3-62A
April 29, 1991

# DATA EVALUATION RECORD

## 2,4-DICHLOROPHENOXYFROPIONIC ACID

## Mutagenicity--*Salmonella typhimurium* Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir
Date: 4/29/91

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Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 362-A
TASK No.: 3-62A
April 29, 1991

**DATA EVALUATION RECORD**

**2,4-DICHLOROPHENOXYPROPIONIC ACID**

**Mutagenicity--Salmonella typhimurium Mammalian Microsome Mutagenicity Assay**

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation
Signature: Nancy E. McCarroll
Date: 4-29-91

I. Cecil Felkner, Ph.D.
Independent Reviewer
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APPROVED BY:

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Signature: K. Whitby
Date: 5/13/91

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)
Signature: K. Clark Swentzel
Date: 5/17/91

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**Salmonella**

**DATA EVALUATION RECORD**

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: 2,4-Dichlorophenoxypropionic acid.

STUDY TYPE: Salmonella/mammalian activation gene mutation assay.

MRID NUMBER: 416468-01.

SYNONYMS/CAS NUMBER: 2,4-DP; Dichlorprop.

SPONSOR: BASF Corp., Research Triangle Park, NC.

TESTING FACILITY: BASF Aktiengesellschaft, Ludwigshafen/Rhein, Federal Republic of Germany.

TITLE OF REPORT: Mutagenicity Testing--2,4-Dichlorophenoxy-propionic Acid in the Ames Test.

AUTHOR: G. Engelhardt.

STUDY NUMBER: 81/0317.

REPORT ISSUED: March 18, 1981.

CONCLUSIONS - Executive Summary:

Five concentrations of 2,4-dichlorophenoxypropionic acid (2,4-DP) ranging from 20 to 5000 µg/plate were evaluated in the Salmonella typhimurium mammalian microsome mutagenicity assay. The test material was cytotoxic in all strains at the two highest doses (2500 and 5000 µg/plate +/-S9) but failed to induce a mutagenic response in S. typhimurium TA1535, TA1537, TA1538, TA98, or TA100. However, the S9-activated assay was conducted with an excessive concentration of S9 (30%) in the S9 reaction mixture. Unless the author can justify the use of a high S9 concentration, the assay should be repeated using the recommended screening concentration (4% S9 in the S9 mix). Additionally, analytical data to support the actual concentrations of the test material used in the assay and a statement of quality assurance were not provided.

The study, therefore, does not fulfill the Guideline requirements for genetic effects, Category I, Gene Mutations.

Study Classification: The study is unacceptable.

**Salmonella**

A. **MATERIALS:**

1. **Test Material:**

| | |
|---|---|
| **Name:** | 2,4-Dichlorophenoxypropionic acid (2,4-DP). |
| **Description:** | Yellowish-brown solid (see Data Evaluation Record 362-B); the chemical formula was provided. |
| **Identification Number:** | 80/356. |
| **Purity:** | ≈95%. |
| **Contaminants:** | None listed. |
| **Solvent used:** | Dimethyl sulfoxide (DMSO). |
| **Other comments:** | The test material was stored at 4°C. No information on solution preparation was provided. |

2. **Control Materials:**

**Negative:** DMSO.
**Solvent/final concentration:** 100 µL/plate
**Positive:** Nonactivation:

| | | |
|---|---|---|
| N-methyl-N'-nitro-N-nitrosoquanidine | 5 µg/plate | TA100, TA1535 |
| 4-Nitro-o-phenylenediamine | 10 µg/plate | TA98, TA1538 |
| 9-Aminoacridine | 100 µg/plate | TA1537 |

**Other:**

**Activation:**
2-Aminoanthracene (2-anthramine) 10 µg/plate all strains.

3. **Activation:** S9 derived from male Sprague Dawley

| | | | |
|---|---|---|---|
| x Aroclor 1254 | x induced | x rat | x liver |
| _____ phenobarbital | _____ noninduced | _____ mouse | _____ lung |
| _____ none | | _____ hamster | _____ other |
| _____ other | | _____ other | |

The S9 liver homogenate was prepared by the performing laboratory. S9 mix composition per milliliter was as follows:

| Component | Concentration |
|---|---|
| $H_2O$ | 0.70 mL |
| $NaH_2PO_4/NaHPO_4$ (pH 7.4) | 100 mM |
| Glucose 6-phosphate | 5 mM |
| NADP | 4 mM |
| $MgCl_2$ | 8 mM |
| KCl | 33 mM |
| S9 | 0.30 mL (30%) |

**Salmonella**

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538; list any others:

5. Test Compound Concentration Used:

a. Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was not performed.

b. Mutation Assay: Five doses (20, 100, 500, 2500, and 5000 µg/plate) were evaluated in both the presence and absence of S9 activation. Four replicate plates were prepared per dose per strain per condition.

B. TEST PERFORMANCE:

Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (__ ) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

Protocol:

Plating Procedures: To tubes containing 2.0-mL volumes of molten top agar, 100 µL of an overnight broth culture of the appropriate tester strain and 100 µL of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated test, 0.5 mL of the S9 cofactor mix was added to tubes containing 2.0 mL of top agar; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner Minimal Medium E, and incubated at 37°C for 48 hours. At the end of incubation, plates were scored for revertant colonies.

Evaluation Criteria: The test material was considered positive if it caused a doubling of the spontaneous mutation rate of any strain; if the increase was dose-related; and if the results were reproducible.

C. REPORTED RESULTS:

Five doses of the test material ranging from 20 to 5000 µg/plate were tested in the presence or absence of S9 activation. Quadruplicate plates were prepared per dose per strain per condition. Average values calculated by our reviewers indicated that cytotoxicity both with and without S9 activation was apparent for all strains at the two highest dose

levels (2500 and 5000 µg/plate). Slightly lower than control revertant colony counts were noted for the majority of strains at 500 µg/plate +/-S9; the reductions were, however, not considered to be indicative of cytotoxicity at this level (Table 1).

Results for lower concentrations (20 and 100 µg/plate +/-S9) indicated that there was no appreciable increase in reversion to histidine prototrophy of any strain at the noncytotoxic levels either with or without S9 activation. Because there was no response in strain TA98 with the nonactivated positive control compound, this portion of the assay was repeated. The representative nonactivated findings from the repeat test with strain TA98 are also presented in Table 1.

The sensitivity of the test system to detect the mutagenic action of the nonactivated positive controls was clearly demonstrated. Although the strains responded to the mutagenic action of the S9-activated positive control (10 µg/plate 2AA), our reviewers noted that both the concentration of 2AA that was used and the percentage of S9 in the S9-cofactor mix (30%) were considerably higher than levels generally required to show assay sensitivity.

Based on the overall findings, the study author concluded that 2,4-DF is not mutagenic in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the results of the S. typhimurium reverse mutation assay with 2,4-DP did not suggest a positive effect. However, the use of 30% S9, as the primary screening concentration is not recommended[1] and may have compromised the sensitivity of the system to detect a potential promutagen. It is possible for a promutagen to be deactivated and/or to be detoxified by high mixed-function oxidase enzyme levels. Although conversion of the promutagen 2AA to an active mutagenic metabolite was demonstrated, the dose of 2AA (10 µg/plate) was higher than is conventionally applied. Using the recommended concentration of S9 in the S9 mix (4%), peak mutagenic activity of 2AA can be achieved at doses ranging from 0.5 to 2.5 µg/plate. It is not clear whether this high level of 2AA was required because of the excessive amount of S9 in the activation mix. We conclude, therefore, that unless the biochemical characteristics of the test material indicate a

[1]Maron, M., and Ames, B.N. 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113:173-215.

**Salmonella**

TABLE 1. Representative Results of the Salmonella typhimurium Mutagenicity Assay with 2,4-Dichlorophenoxypropionic Acid (2,4-DP)

| Substance | Activation | Dose/plate (µg/plate) | Revertants per Plate of Bacterial Tester Strain[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative Control | | | | | | | |
| Dimethyl sulfoxide | - | -- | 14.5 | 4.8 | 13.3 | 19.8[b] | 110.8 |
| | + | -- | 14.0 | 5.5 | 22.8 | 35.3 | 117.0 |
| Positive Controls[c] | | | | | | | |
| MNNG | - | 5.0 | 2187.5 | -- | -- | -- | 2537.5 |
| 4NPA | - | 10.0 | -- | -- | 307.5 | 790.3[b] | -- |
| 9AA | - | 100.0 | -- | 536.5 | -- | -- | -- |
| 2AA | + | 10.0 | 403.8 | 138.0 | 1512.5 | 1667.5 | 2350.0 |
| Test Material | | | | | | | |
| 2,4-DP | - | 500[d] | 12.3 | 4.3 | 10.5 | 17.0[b] | 100.8 |
| | - | 2500 | 6.8 | 2.5[e] | 7.3 | 17.5[b] | 80.5 |
| | - | 5000 | 4.5 | 1.0[e] | 3.8[e] | 11.8[b] | 48.0[e] |
| | + | 500[d] | 9.8 | 6.5 | 19.8 | 35.8 | 99.8 |
| | + | 2500 | 7.3 | 4.8 | 16.5 | 18.0 | 62.0[e] |
| | + | 5000 | 5.5 | 2.0[e] | 10.8 | 12.0 | 37.0[e] |

[a]Average count from quadruplicate plates; calculated by our reviewers.

[b]Results of repeat nonactivated test with strain TA98; the initial assay was repeated owing to the lack of a response with the positive control compound.

[c]Abbreviations used:

MNNG - N-methyl-N'-nitro-N-nitrosoguanidine
4NPA - 4-Nitro-o-phenylenediamine
9AA - 9-Aminoacridine
2AA - 2-Aminoanthracene

[d]Results for lower doses (20 and 100 µg/plate +/-S9) did not suggest a mutagenic effect.

[e]Reduction in the background lawn of growth.

requirement for high enzyme levels, the use of 30% S9 as the primary screening concentration is not an acceptable practice.

We further assess that the lack of analytical data to support the actual test material concentrations used in this assay renders the overall study unacceptable.

It is of note that a previously reviewed S. typhimurium reverse mutation assay with the D-form of 2,4-DP, which was submitted by the sponsor of the currently reviewed study, was also classified as unacceptable on the basis of an excessive concentration of S9 in the S9 mix. (See Data Evaluation Record 249-B, dated December 20, 1989.)

E. QUALITY ASSURANCE MEASURES: A good laboratory practices statement, signed and dated 6 months after completion of this study, was present; however, a quality assurance statement was not included in the report.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 0009-0014.

# APPENDIX A

## Materials and Methods
(CBI pp. 0009-0014)

EPA No.: 68D80056
DYNAMAC No.: 362-B
TASK No.: 3-62B
April 29, 1991

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# DATA EVALUATION RECORD

## 2,4-DICHLOROPHENOXYPROPIONIC ACID

## Mutagenicity--*In vivo* Cytogenetic Assay with Chinese Hamsters

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/29/91





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Guideline Series 84: Mutagenicity
EPA No.: 68D80056
DYNAMAC No.: 362-B
TASK No.: 3-62B
April 29, 1991

008379

# DATA EVALUATION RECORD

## 2,4-DICHLOROPHENOXYPROPIONIC ACID

## Mutagenicity--In vivo Cytogenetic Assay with Chinese Hamsters

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-29-91

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-29-91

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: for
Date: 4-29-91

Karen Whitby, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature:
Date: 5/13/91

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 5/17/91

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DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: 2,4-Dichlorophenoxypropionic acid.

STUDY TYPE: Mutagenicity--In vivo cytogenetic assay with Chinese hamsters.

MRID NUMBER: 41.6468-02.

SYNONYMS/CAS NUMBER: 2,4-DP; Dichloroprop.

SPONSOR: BASF Corp., Research Triangle Park, NC.

TESTING FACILITY: BASF Aktiengesellschaft, Ludwigshafen/Rhein, Federal Republic of Germany.

TITLE OF REPORT: Cytogenetic Investigations of 2,4-DP in Chinese Hamsters--Bone Marrow Chromosome Analysis.

AUTHOR: G. Engelhardt.

STUDY NUMBER: 85/0095.

REPORT ISSUED: April 1, 1985.



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CONCLUSIONS - Executive Summary:

The potential of 2,4-DP to induce chromosome damage was investigated in Chinese hamsters. Groups of 10 animals (5 males and 5 females) received single oral gavage administrations of 47, 280, or 1780 mg/kg of the test material and were sequentially sacrificed 6, 24, and 48 hours following treatment. Toxic signs, which included death in one female, apathy, atony, irregular respiration, squatting posture, and piloerection, were seen in the high-dose group. Similar but less intense toxic effects were also observed in the mid-dose group. These toxicological signs were consistent with those observed in a preliminary acute oral toxicity range-finding study conducted with the test material.

Although the data indicate that an appropriate range of test material doses was evaluated for this in vivo study, no definitive conclusions could be reached regarding the potential clastogenesis of 2,4-DP. Significant ($p < 0.01$) increases in the percentage of aberrant cells were observed in the 1780-mg/kg dose group at the 24- and 48-hour harvest interval. However, these increases did not appear to be time or dose dependent and were limited to the high-dose group. Our ability to independently review the data was further limited for the following reasons:

1. Only aberrations classified as exchanges or cells with pulverized chromosomes or multiple aberrations (i.e., ≥5 aberrations/cell) were identified.

2. Classifying cells with ≥5 aberrations/cell as multiple aberrations does not conform with the generally accepted approach (i.e., multiple aberrations are cells with ≥10 aberrations/cell) and further limits the independent assessment of the data. The type and frequency of specific aberrations is as important a determinant of clastogenesis as is the frequency of cells with aberrations.

3. Mid- and low-dose groups from the 6-hour and 48-hour harvest were not scored; at minimum, the 48-hour harvest cells should have been analyzed. We do, however, concur that the mitotic indices for the high-dose group at 6, 24, and 48 hours posttreatment did not suggest a delay in cell cycling.

4. Since dosing was based on animal weight data, individual body weights should have been included in the report.

However, the findings of an in vivo sister chromatid exchange (SCE) assay in Chinese hamsters administered comparable doses of the test material showed clear dose-related genotoxic effects; the SCE frequencies were significantly ($p < 0.01$) increased in the mid-



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(280 mg/kg) and high-dose (1780 mg/kg) groups (see Data Evaluation Record 362-C). Since SCE induction frequently occurs at doses lower than required to induce chromosomal aberrations, the SCE assay is considered to be a sensitive indicator of clastogenesis.[1] Hence, the SCE assay results provide additional support that 2,4-DP may be a clastogen.

Since definitive conclusions can not be reached, the study does not satisfy Guideline requirements for genetic effects Category II, Structural Aberrations. However, we classify 2,4-DP as presumptively positive.

Study Classification: The study is unacceptable; however, 2,4-DP is classified as presumptively positive.

Recommendations: The study should be repeated with consideration given to the study deficiencies outlined above.

A. MATERIALS:

1. Test Material:

| | |
|---|---|
| Name: | 2,4-Dichlorophenoxypropionic acid (2,4-DP). |
| Description: | Yellowish-brown solid. |
| Identification No.: | 83/48. |
| Purity: | 93.1%. |
| Contaminants: | None listed. |
| Solvent used: | 0.5% aqueous carboxymethyl cellulose (CMC). |
| Other comments: | The test material was stored at 4°C. The homogeneity and stability of the dose solutions were determined analytically. Suspensions of the test material used in this study were prepared immediately prior to use. |

[1]Latt, S.A., Allen, J., Bloom, S.E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., and Wolff, S. (1981) Sister-chromatid exchanges: A report of the Gene-Tox Program. Mutat. Res. 84: 17-62.

2. Control Materials:

Negative/Route of Administration: None.

Vehicle/Final Concentration/Route of Administration: 0.5% CMC was administered once by oral gavage at a dosing volume of 10 mL/kg.

Positive/Final dose(s)/Route of Administration: Cyclophosphamide (CP) was prepared in distilled water and administered once by oral gavage at a dose of 60 mg/kg.

3. Test Compound:

Volume of test substance administered: 10 mL/kg.

Route of administration: Oral gavage.

Dose levels used: 47, 280, and 1780 mg/kg.

4. Test Animals:

a. Species Chinese hamster Strain Not reported Age 7 to 13 weeks Weight Not reported by sex; mean weight: 21.12 g. Source: BASF, Ludwigshafen/Rhein, Federal Republic of Germany .

b. No. animals used per dose:

- Treatment groups: 5 males 5 females/group/sacrifice time.
- Positive control: 5 males 5 females sacrificed 24 hours posttreatment.
- Vehicle control: 10 males 10 females sacrificed 24 hours postadministration.

c. Properly maintained? Yes .

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:
   a. Test Compound
      Dosing: x once ____ twice (24 hr apart)
      ____ other (describe):

Sampling (after last dose): ___x___ 6 hr _____ 12 hr
___x___ 24 hr ___x___ 48 hr _____ 72 hr (mark all that are appropriate)
_____ other (describe):

b. Negative and/or vehicle control
Dosing: ___x___ Once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____12 hr
___x___ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
_____ other (describe):

c. Positive control
Dosing: ___x___ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
___x___ 24 hr _____ 48 hr _____ hr (mark all that are appropriate)
_____ other (describe):

d. Administration of spindle inhibitor
Inhibitor used/dose: Colcemid/3.3 mg/kg

Interval administered before animal killed: 2 hours.

Route of administration ____x___ i.p. _____ other (describe)

2. Tissues and Cells Examined:

___x___ bone marrow _____ other (list):

No. of cells per animal per treatment group examined: 100.

No. of cells per animal per control group examined: 100.

(Note: Mitotic indices were determined from the evaluation of 1500 cells/animal/treatment or control group).

3. Details of Cell Harvest and Slide Preparation:

Groups of five males and five females in the treatment groups were sacrificed by an unspecified method at 6, 24, and 48 hours postexposure to the selected doses of the test material. Animals in the vehicle and positive control groups were sacrificed 24 hours following treatment.

Bone marrow cells were collected from both femurs by aspiration into Hanks' solution. Cells were centrifuged, treated with hypotonic 1% sodium citrate, and fixed in methanol:glacial acetic acid (3:1). Slides were stained with 5% Giemsa and coded.

4. Statistical Evaluation: The data were analyzed for statistical significance at p values of 0.05 and 0.01 by Fisher's Exact test and the Mann-Whitney U-test.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Study: An acute oral toxicity study was performed; the details were not reported. The author stated, however, that deaths were observed in groups receiving doses ≥2150 mg/kg, and clinical signs of toxicity (dyspnea, apathy, atony, tremors, twitching, and piloerection) were seen in animals administered 1780 mg/kg of the test material. The study author, therefore, selected 47, 280, and 1780 mg/kg as the low, intermediate, and high dose, respectively, for the cytogenetic assay.

2. Test Material Analysis: Data presented by the study author from the analysis of test material solutions showed that the actual concentrations of 2,4-DP, were ≈86 to 99% of the target concentrations. The study author stated that the differences between actual and theoretical values were within an acceptable range. Values from duplicate samples analyzed for achieved concentrations were generally comparable, indicating that the test material was uniformly distributed throughout the dosing solutions.

3. Animal Observations: One female in the high-dose group died 2 days after administration of 1780 mg/kg 2,4-DP. Clinical signs of atony, apathy, piloerection, irregular respiration, squatting posture, trembling, and twitching were noted in high-dose animals within 15 minutes of test material administration; some of these signs persisted until the scheduled sacrifice. Animals receiving 280 mg/kg showed similar but less intense toxic signs. The low-dose group (47 mg/kg) appeared to be unaffected by compound treatment. Gross pathological examinations did not reveal any compound-related effects on internal organs.

4. Cytogenetic Assay: Representative results from the cytogenetic assay conducted with 47, 280, and 1780 mg/kg 2,4-DP are presented in Table 1. As shown, analysis of metaphases recovered from high-dose males and females 24 and

TABLE 1. Representative Results of the *in vivo* Cytogenetic Assay in Chinese Hamsters Exposed to 2,4-DP

| Substance | Dose/kg | Exposure Time (hours) | Sex | No. of Animals Examined per Group | No. of Metaphases Examined | Percent Mitotic Index[a] | Total Number of Cells with Aberrations[a,b] | Percent Cells with Aberrations[a,b] | Biologically Significantly Aberrations No./Type[a,c] |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle Control | | | | | | | | | |
| 0.5% Carboxy-methyl cellulose | 10 mL | 24 | M | 9[e] | 900 | 5.82 | 2 | 0.17 | Not identified[d] |
| | | | F | 9[e] | 900 | | 1 | | |
| Positive Control | | | | | | | | | |
| Cyclophosphamide | 60 mg | 24 | M | 3[e] | 300 | 3.69 | 89 | 28.50* | 90E;78M;39P |
| | | | F | 5 | 500 | | 139 | | |
| Test Material | | | | | | | | | |
| 2,4-DP | 1780 mg[f] | 6 | M | 5 | 500 | 3.58 | 2 | 0.50 | Not identified[d] |
| | | | F | 5 | 500 | | 3 | | |
| | | 24 | M | 5 | 500 | 5.73 | 2 | 1.22* | 4M |
| | | | F | 4[e] | 400 | | 9 | | |
| | | 48 | M | 5 | 500 | 7.13 | 8 | 1.11* | Not identified[d] |
| | | | F | 4[e] | 400 | | 2 | | |

[a]Combined data for males and females.

[b]Gaps excluded.

[c]Abbreviations used to identify type and frequency of aberrations combined for both sexes and tabulated by our reviewers.
E -- Exchange
P -- Pulverized Chromosome
M -- Multiple aberrations (≥5 aberrations/cell)

[d]Although a code was presented for aberrations other than those listed above, the report stated that exchanges, pulverized chromosome, or multiple aberrations were not found in these groups.

[e]Metaphases were not scored for two animals in the vehicle control group and two animals in the positive control group; the reason was not provided. One animal in the 24-hour sacrifice of the high-dose group was excluded from the analysis because a marker chromosome was found.

[f]Results from the 24-hour harvest of males and females exposed to the low-(47 mg/kg) or mid-(280 mg/kg) dose were not significantly different from the vehicle control.

[g]One female died prior to the final sacrifice.

*Significantly different from the control ($p<0.01$) by Fisher's Exact test.

48 hours posttreatment revealed that the combined percentage of cells with aberrations for both sexes was significantly ($p < 0.01$) higher than the combined vehicle control group value. The effect was, however, not time related. No significant effects were observed in the high-dose group at the 6-hour harvest interval or in the mid- or low-dose groups harvested 24 hours postexposure. The frequency of numerical aberrations in treatment groups was generally comparable to the vehicle control frequency. The study author stated that the significantly increased percentage of aberrant cells in the 1780-mg/kg dose group at the 24- and 48-hour harvests was "influenced by less than 50% of the animals/group and that more than 50% of the hamsters have an aberration rate in the range of the positive control." The study author did, however, state that "the results do not allow any final conclusions to be drawn regarding the clastogenic activity of the test substance."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the study author that no definitive conclusions can be reached from the bone marrow cytogenetic assay conducted with 47, 280, and 1780 mg/kg 2,4-DP. We further assess that based on the lack of a sizable historical database on the background frequency of chromosome aberrations in Chinese hamsters, we are unable to determine whether the significant increases seen at 24 and 48 hours postexposure to the high dose were artifactual (i.e., resulting from a low vehicle control frequency) or indicative of a weak clastogenic effect. However, the background frequency of aberrant cells in previously reviewed *in vivo* Chinese hamster cytogenetic assays submitted to EPA by the performing laboratory suggest that the combined value for the vehicle control group in the current study is within the reporting laboratory's historical range.

However, clear evidence that 2,4-DP induced dose-related sister chromatid exchange (SCE) in Chinese hamsters *in vivo* was shown in a study conducted with comparable doses of the test material. (See Data Evaluation Record 362-C). These results suggested to our reviewers that the *in vivo* cytogenetic assay findings may not have been artifactual but indicative of clastogenic activity. We conclude, however, that the issue can be resolved only by repeating the study. We also recommend that the specific types of aberrations observed be reported. Further, the reporting laboratory should adopt the generally accepted approach of classifying cells with ≥10 aberrations as multiple aberrations rather than using this classification for cells with ≥5 aberrations. This information could have been helpful in resolving the validity of the increase seen in

the high-dose 24-hour harvest group. Finally, the reporting laboratory should provide individual body weight data since dosing was based on body weight.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated April 9, 1985.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 0011-0024.

# APPENDIX A

## Materials and Methods
(CBI pp. 0011-0024)

EPA No.: 68D80056
DYNAMAC No.: 362-C
TASK No.: 3-62C
April 29, 1991

008379

DATA EVALUATION RECORD

2,4-DICHLOROPHENOXYPROPIONIC ACID

Mutagenicity--In vivo Sister Chromatid Exchange Assay with Chinese Hamsters

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 4/29/91





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**Guideline Series 84: Mutagenicity**
**EPA No.: 68D80056**
**DYNAMAC No.: 362-C**
**TASK No.: 3-62C**
**April 29, 1991**

008379

**DATA EVALUATION RECORD**

**2,4-DICHLOROPHENOXYPROPIONIC ACID**

**Mutagenicity--In vivo Sister Chromatid Exchange Assay with Chinese Hamsters**

REVIEWED BY:

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DATA EVALUATION RECORD 008379

Tox. Chem. No.:
EPA File Symbol:

**STUDY TYPE:** Mutagenicity--In vivo sister chromatid exchange assay with Chinese hamsters.

**MRID NUMBER:** 416468-03.

**TEST MATERIAL:** 2,4-Dichlorophenoxypropionic acid.

**SYNONYMS:** 2,4-DP; Dichloroprop.

**STUDY NUMBER:** 85/0096.

**SPONSOR:** BASF Corp., Research Triangle Park, NC.

**TESTING FACILITY:** BASF Aktiengesellschaft, Ludwigshafen/Rhein, Federal Republic of Germany.

**TITLE OF REPORT:** Cytogenetic Investigations of 2,4-DP in Chinese Hamsters -- Sister Chromatid Exchange.

**AUTHOR:** G. Engelhardt.

**REPORT ISSUED:** March 28, 1985.



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CONCLUSIONS - Executive Summary:

The potential of 2,4-DP to induce sister chromatid exchanges (SCE) was investigated in Chinese hamsters. Groups of 10 animals (5 males and 5 females) received single oral gavage administrations of 47, 280, or 1780 mg/kg of the test material and were sacrificed 24 hours following treatment. Toxic signs which included death, apathy, atony, irregular respiration, squatting posture, and piloerection were seen in the high-dose group. Similar but less toxic effects were also observed in the mid-dose group. These toxicological signs were consistent with those observed in a preliminary acute oral toxicity range-finding study conducted with the test material.

The analysis of metaphases showed a clear dose-related increase in the frequency of SCEs; the findings for the mid- and high-dose groups were significant ($p < 0.01$). We disagree with the study author's claim that the induced response was weak. The intensity of the response could not be determined because conditions were not optimized for SCE detection. The subcutaneous implantation of 5-bromodeoxyuridine (BrdU) pellets into hamster necks in conjunction with compound administration 2 hours postimplantation may have lessened assay sensitivity and, therefore, limited the full expression of the genotoxic response. We, nevertheless, assess that there is sufficient evidence from this study to classify 2,4-DP as positive in this in vivo test system. Based on the overall results, the study satisfies Guideline requirements for Category III, Other Mutagenic Mechanisms.

Study Classification: The study is acceptable; 2,4-DP is positive for the induction of SCEs in Chinese hamsters.

Recommendations: It is suggested that future in vivo SCE assays conducted by the performing laboratory follow the recommended approach for BrdU administration.[1]

[1]Latt, S. A., Allen, J., Bloom, S. E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., and Wolff, S. 1981. Sister-chromatid exchanges: A report of the Gene-Tox Program. Mutat. Res. 87:17-62.



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**In vivo Mammalian SCE**

## A. MATERIALS:

1. Test Material:

| | |
|---|---|
| Name: | 2,4-Dichlorophenoxypropionic acid (2,4-DP) |
| Description: | Yellowish-brown solid (see Data Evaluation Record 362-B); the structural formula was supplied. |
| Batch No.: | 83/48. |
| Purity: | 93.1%. |
| Contaminants: | None listed. |
| Solvent used: | 0.5% aqueous carboxymethyl cellulose (CMC). |
| Other comments: | The test material was stored at 4°C. The homogeneity and stability of the dose solutions were determined analytically. Suspensions of the test material used in this study were prepared immediately prior to use. |

2. Control Materials:

Negative/Route of Administration: None.

Vehicle/Final Concentration/Route of Administration: 0.5% CMC was administered once by oral gavage at a dosing volume of 10 mL/kg.

Positive/Final Concentration/Route of Administration: Cyclophosphamide (CP) was prepared in distilled water and administered once by oral gavage at a dose of 20 mg/kg.

3. Test Compound: Route of administration: Oral gavage.

Dose levels used: 47, 280, and 1780 mg/kg.

4. Test Animals:

a.

| | |
|---|---|
| Species: | Chinese hamster |
| Strain: | Not reported |
| Mean weight: | Not reported by sex; weight range: 27 to 31 g |
| Age: | Not reported |
| Source: | BASF, Ludwigshafen/Rhein, Federal Republic of Germany |

b. No. animals used per dose/group:

Treatment groups: 5 males 5 females/group

Positive control: 5 males 5 females

Vehicle Control: __5__ males __5__ females

c. Properly maintained?: Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
____ other (describe):

b. Negative and/or vehicle control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
____ other (describe):

c. Positive control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
____ other (describe):

d. Administration of spindle inhibitor
Inhibitor used/dose: Colcemid/3.3 mg/kg

Interval administered before animal killed: 2 hours

Route of administration: __X__ i.p. _____ other (describe)

2. Tissues and Cells Examined:

__X__ bone marrow _____ other (list):

No. of cells examined per animal per treatment group: 30.

No. of cells examined per animal per treatment group: 30 (vehicle control group) 10 (positive control group)

Note:

Mitotic indices were not determined.

3. 5-Bromodeoxyuridine (BrdU) Implantation: A 50-mg BrdU tablet was implanted subcutaneously into the neck region of each animal 2 hours prior to administration of the selected test material doses.

4. Details of Cell Harvest and Slide Preparation: Bone marrow cells were collected from both femurs by aspiration into Hanks' solution. Cells were centrifuged, treated with hypotonic 1% sodium citrate, and fixed in methanol:glacial acetic acid (3:1). Slides were stained with Hoechst 33258, rinsed in pH 6.8 buffer, irradiated for 25 minutes, stained in Giemsa, mounted, and coded.

5. Statistical Evaluation: The data were analyzed for statistical significance at p values of 0.05 and 0.01 by a nonparametric one-sided test. The positive control group data were not statistically evaluated.

C. REPORTED RESULTS:

1. Preliminary Toxicity Study: An acute oral toxicity study was performed; the details were not reported. The author stated, however, that deaths were observed in groups receiving doses ≥2150 mg/kg and clinical signs of toxicity (dyspnea, apathy, atony, tremors and twitching, and piloerection) were seen in animals administered 1780 mg/kg of the test material. Similar but less intense toxic signs were seen in animals receiving 280 mg/kg/day 2,4-DP. The study author, therefore, selected 47, 280, and 1780 mg/kg as the low, intermediate, and high dose, respectively, for the SCE assay.

2. Test Material Analyses: Data presented by the study author from the analysis of test material solutions showed that the actual concentrations of 2,4-DP were 84 to 92% of the target concentrations. The study author stated that the differences between actual and theoretical values were within an acceptable range. Values from duplicate samples analyzed for achieved concentrations were generally

comparable, indicating that the test material was uniformly distributed throughout the dosing solutions.

3. Animal Observations: Clinical signs of atony, apathy, piloerection, irregular respiration, and squatting posture were noted in high-dose animals within 15 minutes of test material administration; these signs persisted until the scheduled sacrifice. Animals receiving 280 mg/kg showed similar but less intense toxic signs. The low-dose group (47 mg/kg) appeared to be unaffected by compound treatment. Gross pathological examinations did not reveal any compound-related effects on internal organs.

4. SCE Assay: Results from the SCE assay conducted with 47, 280, and 1780 mg/kg 2,4-DP are presented in Table 1. As shown, the analysis of metaphases for the three treatment groups revealed a dose-related increase in the frequency of SCEs. The mean data combined for both sexes were significantly increased ($p < 0.01$) in the mid- and high-dose groups. The data further suggest that the genotoxic effect was more pronounced in the females than in the males.

   Based on the results, the study author concluded that "2,4-DP has a weak SCE-inducing activity in vivo on bone marrow cells of Chinese hamsters."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the study author that 2,4-DP was positive in this in vivo SCE assay. However, we disagree with the conclusion that the response was weak. The intensity of the response cannot be fully determined for this study because conditions were not optimized for SCE induction. The method of BrdU implantation (neck) and the administration of the test material 2 hours post-BrdU implantation may have biased the results by reducing assay sensitivity. The standard procedure requires BrdU tablet implantation in the lateral abdominal region. Using this method, the test material is administered 8 hours after tablet implantation. The 8-hour interval permits the slow release of BrdU and the collection of second-cycle metaphases.[2]

[2]Latt et al., 1981.

TABLE 1. Results of the In vivo Sister Chromatid Exchange Assay in Chinese Hamsters Treated with 2,4-DP

| Substance | Dose/kg | No. of Animals Exposed per Group | No. of Animals Analyzed per Group | No. of Metaphases Analyzed per Group | Average[a] SCEs per Sex | Mean Group[b] SCEs ±S.D. |
|---|---|---|---|---|---|---|
| **Vehicle Control** | | | | | | |
| 0.5% Carboxymethyl | 10 mL | 5 M | 5 M | 150 | 3.18 | 3.31 ± 0.501 |
| | 10 mL | 5 F | 5 F | 150 | 3.46 | |
| **Positive Control** | | | | | | |
| Cyclophosphamide | 20 mg | 5 M | 5 M | 50 | 35.44 | 31.91 ± 5.164 |
| | 20 mg | 5 F | 5 F | 50 | 28.40 | |
| **Test Material** | | | | | | |
| 2,4-DP | 47 mg | 5 M | 5 M | 150 | 3.34 | 3.54 ± 0.517 |
| | 47 mg | 5 F | 5 F | 150 | 3.76 | |
| | 280 mg | 5 M | 4 M[c] | 120 | 3.88 | 4.67 ± 0.981* |
| | 280 mg | 5 F | 5 F | 150 | 5.30 | |
| | 1780 mg | 5 M | 5 M | 150 | 6.72 | 7.32 ± 1.194* |
| | 1780 mg | 5 F | 4 F[c] | 120 | 8.05 | |

[a]Averaged by our reviewers.

[b]Minor differences between the reported values and the recalculations of the data by our reviewers were noted. These differences, however, did not affect the outcome of the study.

[c]Insufficient number of differentially stained metaphases; therefore, the slides from these animals were not scored.

*Significantly higher than the vehicle control ($p < 0.01$) by nonparametric, one-sided test.

Although mitotic indices were not determined in this assay, the data presented from an in vivo cytogenetic study with comparable doses of 2,4-DP did not suggest adverse effects on the cell-cycling time of Chinese hamster bone marrow cells (see Data Evaluation Record 326-B). We assess, therefore, that there is sufficient evidence from this study to conclude that 2,4-DP is genotoxic in this in vivo test system.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated April 9, 1985.

F. CBI APPENDIX: Appendix A, Material and Methods, CBI pp. 0012-0021.

# APPENDIX A

Materials and Methods
(CBI pp. 0012-0021)