12-15-03

# DATA EVALUATION RECORD

PXTS (Polymeric Xylenol Tetrasulfide)
MRID 46062619

*In vitro* Cytogenetic (Chromosome Aberration) Assay
OPPTS 870.5375

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting
9300 Lee Highway
Fairfax, VA 22031

Under Subcontract to

Versar
6850 Versar Center
P.O. Box 1549
Springfield, VA 22151

| | | |
|---|---|---|
| Principal Reviewer | *Jinny Ha, M.H.S.* | Date 12/9/03 |
| Independent Reviewer | *Nikki Maples, M.S.* | Date 12/9/03 |
| ICF Program Manager | *Kara Altshuler, Ph.D.* | Date 12/10/03 |
| Versar Program Manager | *Linda Phillips, Ph.D.* | Date 12/15/03 |

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EPA WAM: Killian Swift, Ph.D.

This review may have been altered by EPA subsequent to the contractors' signatures above.

**EPA Reviewer:** Tim McMahon **Signature:** ________
**Senior Toxicologist, Antimicrobial Division (7510C)** **Date** 7/1/04

**DATA EVALUATION RECORD**

**STUDY TYPE:** *In vitro* Mammalian Cytogenetics, chromosome aberration, in Chinese Hamster Ovary Cells Assay OPPTS 870.5375 [§84-2]; OECD 473.

**PC CODE:** 006929 **DP BARCODE:** D299112

**TEST MATERIAL (PURITY):** Polymeric xylenol tetrasulfide

**SYNONYMS:** PXTS

**CITATION:** Murli, H. (2002) Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Polymeric Xylenol Tetrasulfide (PXTS). Covance Laboratories Inc., Vienna, VA. Laboratory Study Number 23134-0-437OECD. February 7, 2002. MRID 46062619. Unpublished.

**SPONSOR:** Akzo Nobel Functional Chemicals, LLC
5 Livingstone Avenue
Dobbs Ferry, New York 10522

**EXECUTIVE SUMMARY:** In a mammalian cell cytogenetics (chromosome aberration) assay (MRID 46062619), Chinese hamster ovary cells (CHO) were exposed to PXTS (batch # 6/lot # 1685-11-1) diluted with DMSO. Concentrations of 0, 1.99, 2.84, 4.05, 5.78, 8.26, 11.8, 16.8, 24.0, 34.3, 49.0, 70.0, 100, 141, 188, and 250 µg/mL (8.26, 11.8, 16.8, and 24.0 µg/mL, -S9, or 16.8, 24.0, 34.3, and 49.0 µg/mL, +S9, were analyzed for aberrations) were administered to CHO cell cultures for 3 hours, both with and without metabolic activation from livers of Aroclor 1254-induced rats. In a confirmatory assay with an extended exposure time of 17.5 hours, -S9, CHO cells were also treated with 0, 0.785, 1.57, 3.13, 6.25, 9.38, 12.5, 18.8, and 25.0 µg/mL, (concentrations of 1.57, 3.13, 6.25, and 9.38 µg/mL were scored for aberrations). Activated cultures were exposed to doses of 0, 6.25, 12.5, 25.0, 37.5, 50.0, and 75.0 µg/mL (with doses of 6.25, 12.5, 25.0, and 37.5 µg/mL scored for aberrations) for 3 hours in the confirmatory assay. Cells were harvested approximately 20 hours after the start of treatment and the following endpoints were measured: mitotic index, endoreduplication, polyploidy, and percentage of cells with chromosomal aberrations.

PXTS was tested up to a precipitating concentration of 250 µg/mL, ±S9. A statistically-significant increase in aberrations (excluding gaps) was observed at 49.0 µg/mL, +S9, with a slight dose-response trend. In the confirmatory assay, however, toxicity excluded doses higher

slight dose-response trend. In the confirmatory assay, however, toxicity excluded doses higher than 37.5 µg/mL. A slight dose-response trend was observed with an increasing complexity of the chromosomal aberrations of simple breaks to chromatid and chromosome exchanges as observed in the initial test. The response was considered an epigenetic effect and was considered negative. Statistically-significant increases in endoreduplicated cells were noted in cultures treated with 11.8 µg/mL, -S9, and at all analyzed concentrations, +S9, in the initial assay. The results of the confirmatory assay, using a similar range of concentrations, were negative. The ability of the test article to induce inhibition of cell cycle, indicated by a increase in endoreduplicated cells, was considered to be negative due to the lack of a reproducible response. The test article did not induce a significant, dose-dependent increase in cells with chromosomal aberrations or polyploids. The positive controls induced the appropriate response. **There was no evidence of chromosome aberrations induced over background. The compound was considered negative in this *in vitro* chromosome aberration assay.**

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline for the *in vitro* mammalian cytogenetics, chromosome aberration assay OPPTS 870.5375; OECD 473 for *in vitro* cytogenetic mutagenicity data.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance were provided, but Data Confidentiality statements were not provided.

# I. MATERIALS AND METHODS

## A. MATERIALS:

**1. Test Material:** PXTS

| | |
|---|---|
| **Description:** | Black solid (stability was reportedly the responsibility of the Sponsor). |
| **Lot/Batch #:** | Lot # 1985-11-1 and Batch # 6 |
| **Purity:** | Not reported (reportedly the responsibility of the Sponsor). |
| **CAS # of TGAI:** | Not available |
| | Structure not available |
| **Solvent Used:** | Dimethyl sulfoxide (DMSO) |

**2. Control Materials:**

| | |
|---|---|
| **Negative control:** | Culture medium (McCoy's 5a) |
| **Solvent control (final conc'n):** | DMSO (10 µL/mL) |
| **Positive control:** | Nonactivation: Mitomycin C (0.750 and 1.50 µg/mL [3.0 hours]; and 0.200 and 0.400 µg/mL [17.5 hours])/$H_2O$) |
| | Activation: Cyclophosphamide (5.00 and 10.0 µg/mL/$H_2O$) |

**3. Activation:** S9 derived from

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| X | induced | X | Aroclor 1254 (500 mg/kg) | X | Rat (species not stated) | X | Liver |
| | non-induced | | Phenobarbitol | | Mouse | | Lung |
| | | | None | | Hamster | | Other |
| | | | Other | | Other | | |

**S9 mix composition:** The S9 homogenate (Lot 1230) was purchased from Molecular Toxicology, Inc. Prior to use, the S9 homogenate was mixed with either sucrose or potassium chloride and stored at ≤-60°C. Fresh S9 mix was prepared prior to each use and contained S9 homogenate (1.5%), 1.8 mM $NADP^+$, and 10.5 mM isocitric acid.

**4. Test cells:** mammalian cells in culture

| | |
|---|---|
| | V79 cells (Chinese hamster lung fibroblasts) |
| | Human lymphocytes |
| X | Chinese hamster ovary (CHO) cells (CHO-WBL) |

Media: McCoy's 5a (supplemented with 10% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/mL penicillin G, and 100 µg/mL streptomycin)

| | | |
|---|---|---|
| Properly maintained? | [X] Yes | [ ] No |
| Periodically checked for *Mycoplasma* contamination? | [X] Yes | [ ] No |
| Periodically checked for karyotype stability? | [X] Yes | [ ] No |

**5. Test compound concentrations used:**

Toxicity Assay (3 hour treatment)

Nonactivated conditions: 1.99, 2.84, 4.05, 5.78, 8.26, 11.8, 16.8, 24.0, 34.3, 49.0, 70.0, 100, 141, 188, and 250 µg/mL

| | |
|---|---|
| Activated conditions: | 1.99, 2.84, 4.05, 5.78, 8.26, 11.8, 16.8, 24.0, 34.3, 49.0, 70.0, 100, 141, 188, and 250 µg/mL |
| Mutagenicity Assay–Doses Evaluated (3- hour treatment) | |
| Nonactivated conditions: | 8.26, 11.8, 16.8, and 24.0 µg/mL |
| Activated conditions: | 16.8, 24.0, 34.3, and 49.0 µg/mL |
| Toxicity Assay (17.5 hour treatment) | |
| Nonactivated conditions: | 0.785, 1.57, 3.13, 6.25, 9.38, 12.5, 18.8, and 25.0 µg/mL |
| Activated conditions: | 6.25, 12.5, 25.0, 37.5, 50.0, and 75.0 µg/mL |
| Mutagenicity Assay–Doses Evaluated (17.5-hour treatment) | |
| Nonactivated conditions: | 1.57, 3.13, 6.25, and 9.38 µg/mL |
| Activated conditions: | 6.25, 12.5, 25.0, and 37.5 µg/mL |

## B. TEST PERFORMANCE

**1. Preliminary Cytotoxicity Assay:** A preliminary cytotoxicity assay was not conducted; however, cytotoxicity was included as a part of the initial and repeated mutagenicity tests with 6-8 exposure groups. Cultures demonstrating low cytotoxicity (based on relative confluency) were evaluated for mutagenicity. Changes in cell monolayer confluency were based on visual observations, which were made prior to the harvest of the metaphase cells. Mitotic or dead cells were also evaluated.

**2. Cytogenetic Assay:**

| **a. Cell exposure time:** | Test Material | Solvent Control | Positive Control |
|---|---|---|---|
| Non-activated: | 3 or 17.5 h | 3 or 17.5 h | 3 or 17.5 h |
| Activated: | 3 h | 3 h | 3 h |

**b. Spindle inhibition**

| | |
|---|---|
| Inhibitor used/concentration: | Colcemid/0.1 µg/mL |
| Administration time: | 2 hours (before cell harvest) |

| **c. Cell harvest time after termination of treatment:** | Test Material | Solvent Control | Positive Control |
|---|---|---|---|
| Non-activated: | ~20 h | ~20 h | ~20 h |
| Activated: | ~20 h | ~20 h | ~20 h |

**d. Details of slide preparation:** Cells were harvested by trypsinization, swollen with KCl (75 mM), fixed in methanol:glacial acetic acid (3:1 [v:v]), and dropped onto slides. Slides containing harvested cells were air-dried, stained with 5% Giemsa, air-dried again and permanently mounted.

**e. Metaphase analysis:**
No. of cells examined per dose: 200 cells/dose; 25 cells/positive control (single dose); 1000 cells/treatment dose and vehicle control analyzed for mitotic indices; 100 metaphases/treatment dose and in the vehicle controls analyzed for polyploidy

| | | |
|---|---|---|
| Scored for structural? | X Yes | No |
| Scored for numerical? | X Yes (polyploids and endoreduplication) | No |
| Coded prior to analysis? | X Yes | No |

**f. Evaluation criteria:** Cells of good morphology with 21±2 centromeres/cell were analyzed. A positive response was defined as a significant ($p \leq 0.01$) dose-dependent increase in the number of cells with chromosomal aberrations for one or more concentrations. A negative response was defined as an absence of a significant increase in cells with chromosomal aberrations at any concentration. For an assay to be considered valid, the following criteria must be met: 1) at least 3 analyzable doses; 2) an independent assay was performed; 3) the negative and vehicle control cultures must contain less than 5% aberrant cells; 4) the positive control must produce a significant ($p \leq 0.01$) increase in aberrant cells; 5) the high dose must either be slightly higher than the solubility limit in culture medium or reduce the mitotic index by approximately 50%.

**g. Statistical analysis:** The statistical analyses used in the assay were the following: Cochran-Armitage test for a linear trend and Fisher's Exact Test to compare the percentage of cells with aberrations (treated vs. control). These analyses were appropriate.

**II. REPORTED RESULTS**

**A. PRELIMINARY CYTOTOXICITY ASSAY:** A preliminary cytotoxicity assay was not performed; however, cytotoxicity determination was included in the mutagenicity tests. The test article was evaluated for solubility and was determined to be insoluble in water, ethanol, and acetone. The solvent of choice was DMSO, which achieved a maximum solubility of 100 µg/mL in culture medium. The designated high dose was 250 µg/mL, which is slightly above the solubility limit in culture medium.

**B. CYTOGENETIC ASSAY:** Summary data for both the initial and confirmatory assays are presented in Table 1 below. For the initial assay, cells were exposed to 1.99-250 µg/mL. Precipitation reportedly was noted at 250 µg/mL, ±S9. Data concerning doses of 1.99-2.84 and 70.0-250 µg/mL, -S9, and 1.99-5.78 and 100-250 µg/mL, +S9, were not presented. The absence of cells or a monolayer was reportedly noted at 70.0, 100, 141, 188, and 250 µg/mL, -S9. Reductions in both the confluence of the monolayer and in the number of mitotic cells were observed at 49.0 µg/mL (approximately 85% reduction in cell monolayer confluence) and at 5.78, 11.8, 8.26, 16.8, 24.0, and 34.3 µg/mL (approximately 15% reduction in cell monolayer confluence). Concentrations of 8.26, 11.8, 16.8, 24.0, 34.3, and 49.0 µg/mL displayed reduced mitotic indices as compared to controls by 25, 35, 47, 61, 61, and 92%, respectively. Therefore, only cultures treated with 8.26, 11.8, 16.8, and 24.0 µg/mL were evaluated for chromosomal

aberrations. A slight increase (approximately 5%) in endoreduplication was observed at 11.8 µg/mL, which was attributed to 0% endoreduplication in the vehicle control. This was considered to be a "statistical anomaly" by the study authors; further, there were no statistically significant increases at adjacent doses. No other concentrations induced an increase in chromosomal aberrations, polyploids, or endoreduplication.

For the initial assay, a >90% reduction or total absence in cell monolayer was reportedly noted at 100, 141, 188, and 250 µg/mL, +S9. Reductions in both the confluence of the monolayer and in mitotic cells were observed at 49.0 and 70.0 µg/mL (approximately 45% reduction in cell monolayer confluence) and at 34.3 µg/mL (approximately 15% reduction in confluency). Doses of 24.0, 34.3, 49.0, and 70.0 µg/mL reduced the mitotic indices compared to the vehicle control by 26, 42, 52, and 82%, respectively. Cultures treated with 16.8, 24.0, 34.3, and 49.0 µg/mL were evaluated for aberrations. A concentration of 49.0 µg/mL induced a significant increase in aberrant cells. A dose-dependent increase in the percentage of cells with structural chromosome aberrations with gaps was observed, but the significance of this is minimal. No other dose induced an increase in aberrations or polyploidy. Significant increases in endoreduplicated cells were observed for all analyzed concentrations.

In the confirmatory assay, a 57% reduction in the monolayer confluence was noted at 25.0 µg/mL, -S9. Doses of 18.8 and 12.5 µg/mL reduced the monolayer confluence by approximately 30 and 14%, respectively. Concentrations of 6.25, 9.38, 12.5, 18.8, and 25.0 µg/mL reduced the mitotic indices as compared to controls by 27, 63, 81, 100, and 99%, respectively. Doses of 1.57, 3.13, 6.25, and 9.38 µg/mL were analyzed for chromosomal aberrations. These concentrations did not induce a significant increase in the frequency of cells with chromosomal aberrations, polyploids, or endoreduplication.

For the confirmatory assay, +S9, reductions in monolayer confluence of >90%, 43%, 43%, and 15% were observed in cultures treated with 75.0, 50.0, 37.5, and 25.0 µg/mL, respectively. Mitotic indices were reduced by 45, 73, 81, and 100% in cultures treated with 25.0, 37.5, 50.0, and 75.0 µg/mL, respectively. Cultures analyzed for mutagenicity (6.25, 12.6, 25.0, and 37.5 µg/mL) did not exhibit a significant increase in aberrant cells, polyploids, or endoreduplication. The positive controls produced strong positive results, confirming the sensitivity of the assay.

**Table 1. Summary of Chromosomal Assay Results[a]**

| Concentrations (µg/mL) | Average mitotic index (%) | Endoreduplicated cells (number) | Polyploid Cells (number) | Chromosomal Aberrant Cells (%) |
|---|---|---|---|---|
| **Initial Assay, -S9** | | | | |
| Negative Control | 12.5 | 0.0 | 8.5 | 0.0 |
| Vehicle Control | 12.4 | 0.0 | 10.0 | 0.5 |
| 8.26 | 9.3 | 1.0 | 11.5 | 0.5 |
| 11.8 | 8.0 | 5.0* | 7.0 | 1.0 |

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| Concentrations (µg/mL) | Average mitotic index (%) | Endoreduplicated cells (number) | Polyploid Cells (number) | Chromosomal Aberrant Cells (%) |
|---|---|---|---|---|
| 16.8 | 6.6 | 2.5 | 10.5 | 0.0 |
| 24.0 | 4.8 | 3.0 | 12.0 | 2.5 |
| Positive Control | - | 0.0 | 2.5 | 71.0* |
| **Initial Assay, +S9** | | | | |
| Negative Control | 12.6 | 0.5 | 11.0 | 0.5 |
| Vehicle Control | 14.9 | 1.0 | 13.5 | 0.0 |
| 16.8 | 14.2 | 8.0* | 6.0 | 1.0 |
| 24.0 | 11.1 | 11.0* | 7.0 | 0.5 |
| 34.3 | 8.6 | 11.5* | 13.0 | 3.0 |
| 49.0 | 7.2 | 9.0* | 17.0 | 4.5* |
| Positive Control | - | 1.0 | 2.0 | 44.0* |
| **Confirmatory Assay, -S9** | | | | |
| Negative Control | 7.9 | 0.0 | 0.0 | 1.5 |
| Vehicle Control | 7.8 | 0.0 | 0.5 | 0.5 |
| 1.57 | 9.7 | 0.0 | 0.5 | 1.0 |
| 3.13 | 9.1 | 0.0 | 2.0 | 0.5 |
| 6.25 | 5.7 | 0.5 | 1.0 | 1.5 |
| 9.38 | 2.9 | 0.0 | 2.0 | 2.0 |
| Positive Control | - | 0.0 | 0.0 | 60.0* |
| **Confirmatory Assay, +S9** | | | | |
| Negative Control | 11.4 | 0.0 | 0.5 | 0.0 |
| Vehicle Control | 13.9 | 0.5 | 0.5 | 0.5 |
| 6.25 | 17.1 | 0.0 | 0.0 | 0.5 |
| 12.5 | 14.3 | 1.0 | 0.0 | 1.0 |
| 25.0 | 7.6 | 5.0 | 1.5 | 2.0 |
| 37.5 | 3.8 | 1.5 | 2.0 | 4.0 |
| Positive Control | - | 1.5 | 1.5 | 35.0* |

[a] Data obtained from pp. 19-26; * $p \leq 0.01$

## III. DISCUSSION and CONCLUSIONS

**A. INVESTIGATORS' CONCLUSIONS:** Since the confirmatory assay results did not demonstrate a similar increase in endoreduplicated cells as observed in the initial mutagenicity trial, the increases in endoreduplicated cells observed in the initial assay were considered debatable. No reproducible increase in structural or numerical chromosomal aberrations in CHO cells was observed, ±S9; therefore, based on these findings, PXTS was considered non-mutagenic.

**B. REVIEWER COMMENTS:** The concentrations that induced an increase in endoreduplicated cells in the initial assay, 16.8-49.0 µg/mL, +S9, were reevaluated with a modified range of concentrations (6.25-37.5 µg/mL), as suggested by guidelines. The negative results in the confirmatory assay would have been more persuasive if a wider range of concentrations or a higher percentage of S9 had been used. Higher doses were too toxic for cytogenetic evaluation, however, as they caused unacceptably high decreases in mitotic indices.

In the three hour exposure time experiments, a significant increase in the percentage of cells with chromosomal aberrations (4.5% treated vs. 0.0% in controls) was noted in the presence of S9 at 49.0 µg/mL. This concentration was not reevaluated in the confirmatory assay due to toxicity. The highest exposure concentration used in the confirmatory assay, 37.5 µg/mL, caused a non-significant increase in percentage of cells with chromosomal aberrations (10.5% vs. 2.5% in the vehicle control). Our reviewers noted the aberrations increased in complexity from simple breaks to chromatid and chromosome exchanges, which was also observed in the initial test. However, concentrations causing these effects also resulted in decreases in mitotic index indicative of cellular toxicity.

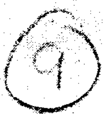
In the 17.5 hour exposure time experiment, no increase in chromosomal aberrations over control was noted, although the increased exposure time was not conducted in the presence of S9, only in the absence of S9.

Historical control data provided in the study report indicate that for either 3 hour or continuous treatment of vehicle controls, the % of cells with aberrations ranges from 0.00-4.50%. Thus, the increase in aberrations in treated cells was also outside of historical control range (page 27).

**C. STUDY DEFICIENCIES:**

Minor Deficiencies

- The purity, expiration date, and other pertinent information concerning the identity of the test article was not provided. This was reportedly the responsibility of the sponsor.
- Homogeneity, stability, and concentration of the test formulations were not verified prior to administration to the test cultures.
- The individual results of all evaluated concentrations were not included in the data tables.
- A protocol deviation was reported on page 16 in which $0.734 \times 10^6$ cells/flask were used which is below protocol [$\sim 1.2 \times 10^6$ cells/flask]. This was due to a low number of cells



available for seeding and probably would not have compromised the study results.

**D. STUDY CLASSIFICATION:**

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline [*In vitro* mammalian cytogenetics test] OPPTS 870.5375.