12-15-03

DATA EVALUATION RECORD

PXTS (polymeric xylenol tetrasulfide)
MRID 46062622

Unscheduled DNA Synthesis in Mammalian Cells in Culture
OPPTS 870.5550

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	*Karg Altshuler, Ph.D.*	Date 12/10/03
Versar Program Manager	*Linda Phillips, Ph.D.*	Date 12/15/03

Contract Number: 68-W-01-036
Work Assignment No.: 0248.3000.002.02 TAF 2-2-21
EPA WAM: Killian Swift, Ph.D.

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

(1)

EPA Reviewer: Tim McMahon
Senior Toxicologist, Antimicrobials Division (7510C)

Signature:
Date 7/11/07

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures; OPPTS 870.5550 (*in vitro*) [§84-2]; OECD 482 (*in vitro*)

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

TEST MATERIAL (PURITY): Polymeric xylenol tetrasulfide (100% a.i.)

SYNONYMS: PXTS

CITATION: Cifone, MA. (2002) Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with Polymeric Xylenol Tetrasulfide (PXTS). Covance Laboratories Inc., Vienna, VA. Laboratory report number 23134-0-447. January 21, 2002. MRID 46062622. Unpublished.

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

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID 46062622), primary rat hepatocyte cultures were exposed to PXTS (100% a.i., batch # 6 and lot # 1685-11-1) in DMSO at concentrations of 0, 0.250, 0.50, 1.00, 2.50, 5.00, and 10.00 µg/mL for approximately 18.8 hours.

PXTS was tested up to the precipitating concentration of 10.0 µg/mL. Five replicate cultures were prepared from a perfused Fischer rat liver for each analyzed concentration. Both cytotoxicity and unscheduled DNA synthesis were evaluated at each dose level. Viability of treated cultures was generally comparable to the vehicle controls, with the exception of the highest dose, which caused a 21% decrease. The test article did not induce an increase in unscheduled DNA synthesis at any of the dose levels analyzed. The positive controls induced the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by nuclear silver grain counts, was induced. Therefore, the test article was negative in this UDS assay.**

(2)

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482 for other genotoxic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: PXTS (polymeric xylenol tetrasulfide)

Description:	Black solid, must be stored at ambient temperature
Lot/Batch #:	Lot # 1685-11-1 and Batch # 6
Purity:	100% a.i.
Compound Stability:	Not provided
CAS # of TGAI:	Not available
	Structure not available
Solvent Used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	DMSO	
Solvent:	DMSO	**Concentration:** 10 mg/mL
Positive control /solvent:	2-Acetylaminofluorene/DMSO	**Concentration:** 0.100 µg/mL

3. Test compound concentrations used: For the concurrent cytotoxicity test, 15 concentrations ranging from 0.00250 to 100 µg/mL were administered. For the UDS assay, concentrations of 0, 0.250, 0.500, 1.00, 2.50, 5.00, and 10.00 µg/mL were evaluated.

4. Media: The base media consisted of Williams' Medium E, 2 mM L-glutamine, 100 µg/mL streptomycin sulfate, and 150 µg/mL gentamicin (WMEI). The base media was supplemented with 10% fetal bovine serum for hepatocyte cultures (WME+). Cultures were labeled with WMEI containing 10 µCi/mL ^{3}H-TdR at 40 to 60 Ci/mMole (WME-treat).

5. Test Cells: Primary cultures of rodent hepatocytes were obtained from the liver of an adult male Fischer rat (Harlan Sprague Dawley, Inc., Frederick, MD).

6. Cell Preparation:

a. Perfusion Technique: The liver was perfused *in situ* with Hank's Balanced Salt Solution (HBSS), 0.5 mM ethyleneglycol-bis(β-aminoethyl ether)-N, N-tetraacetic acid (EGTA), and 50 mM HEPES buffer (pH 7.2) mixture for 4 minutes, followed by the base media supplemented with 50 to 100 units/mL of collangenase (WMEC) for 9 minutes.

b. Hepatocyte Harvest/Culture Preparation: The liver tissue was mechanically dispersed and cells were collected by sedimentation followed by centrifugation, and resuspended in WME+. Following viability determinations, approximately 0.5 x 10^6 cells in 3 mL were plated (five plates per dose) and were cultured on plastic coverslips in petri dishes. Cultures were maintained at 35-37.5°C and 4-6% CO_2. Cells were allowed to attach for 2 hours and any unattached cells were removed after this period. Fresh culture medium was added and the study was initiated approximately 3 hours later. Cells (5 cultures/treatment) were treated for ~19 hours with the base culture medium with 10 µCi/mL ^{3}H-TdR at 40-60 Ci/mmol (WME-treat) and the appropriate test article concentration, vehicle, or positive control.

B. TEST PERFORMANCE

1. Cytotoxicity Assay: Two of the five replicate cultures were used for cytotoxicity determinations. After the treatment period, cultures were refed with base culture medium and allowed to incubate. Approximately 22.8 hours after the initiation of the assay, cellular morphology and cell survival were assessed. Additionally, the Trypan blue dye exclusion test was conducted for viability determinations.

2. UDS Assay: Three of the culture replicates were used for UDS analysis. The culture medium was removed and freshly isolated hepatocytes were treated with various concentrations of the test article in culture medium (approximately 2.5 mL) for 18.8 hours. At the end of the treatment period, the designated UDS assay cultures were rinsed and refed with fresh culture medium containing 1 mM thymidine as a cold chase. Cells were then incubated with 1% sodium citrate for 8 to 12 minutes, fixed, dried, and mounted onto glass slides.

a. Treatment: The five replicate cultures were exposed to 15 different concentrations, ranging from 0.00250 to 100 µg/mL for 18.8 hours; however, only six concentrations, ranging from 0.250 to 10.0 µg/mL, were analyzed for UDS.

b. Preparation of Autoradiographs/Grain Development: Slides were dipped in autoradiographic emulsion (Kodak NTB2) and water, dried, and stored in boxes away from light for 6 days at 2 to 8°C with a desiccant. The emulsion-coated slides were then developed with Kodak D19 and Kodak Rapid Fixer, and stained with a modified hematoxylin and eosin procedure.

c. Grain Counting: Slides were coded to prevent bias. Only "normally-appearing" nuclei were counted. The net grains per nucleus was determined for 50 randomly selected cells on each coverslip; approximately 150 cells per dose (50 from each triplicate coverslip) were examined. Cells undergoing DNA replication (S-phase), represented by blackened nuclei with numerous grains, were not included in the count. The net grains per nucleus was defined as the number of nuclear grains minus the spontaneous background (average number of grains in three nuclear-sized cytoplasmic areas adjacent to each nucleus). The percentage of cells in S-phase were reported.

e. Evaluation Criteria: Criteria for a positive response were based on statistical analysis of laboratory historical control data (provided on pages 22-23 of the study report). For a response to be considered positive, the following criteria must be met: 1) the mean net nuclear grain count must exceed 2.65 (at least five grains per nucleus above the control) and/or 2) at least 16.67% (10% increase relative to the control) of nuclei must have at least five net grains over the control values. In addition, there must be a reproducible, dose-dependent increase in UDS for at least two consecutive dosages. Cases where UDS increases in a dose-dependent manner, and then decreases due to toxicity at higher doses were still considered to be dose-dependent positive responses. A negative response was defined as the absence of a reproducible, dose-dependent increase in mean net nuclear grain count. Judgement of the study director was used for equivocal results. The criteria for a valid assay were as follows: 1) viability of the primary hepatocytes must be above 50%; 2) monolayer culture used for the cytogenetic assay must have ≥ 70% viability; 3) nuclear count for vehicle control must be -5.00 to 1.00 and ≤ 10% cells should contain ≥ 5 net nuclear count; 4) the highest analyzed dose must approach excessive toxicity, test article solubility, or be 5 mg/mL; 5) at least six analyzable doses must be available.

f. Statistical Analysis: Significance of nuclear labeling results were statistically analyzed using the procedures outlined in Casciano and Gaylor (1983)[1]. A review of this published study would be necessary to determine the acceptability of the statistical analysis approach.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: A preliminary cytotoxicity test was not conducted, but was part of the cytogenetic assay. Cells were treated with PXTS at a concentration range of 0.00250-100 µg/mL based on solubility; however, data pertaining to doses not evaluated for the UDS assay were not reported. Doses ≥ 25.0 µg/mL were reportedly excessively toxic. The high dose (10.0 µg/mL) was limited by solubility. The analyzed dosages (0.250, 0.50, 1.00, 2.50, 5.00, and 10.0 µg/mL) selected for the UDS assay were noncytotoxic. Viability of cultures treated with 0.250, 0.50, 1.00, 2.50, 5.00, and 10.0 µg/mL of PXTS after ~19 hours were 101.4, 95.1, 100.5, 104.1, 92.4, and 79.1% of the vehicle control, respectively.

B. UDS ASSAY: Triplicate cultures treated with 0.250, 0.500, 1.00, 2.50, 5.00, or 10.0 µg/mL were evaluated for UDS; approximately 150 cells/dose (50 cells per replicate coverslip) were counted. The mean net nuclear grains, percentage of cells with ≥ five net nuclear grains, mean cytoplasmic grains, viability after the treatment period, and the percentage of S-phase cells were reported. The results are presented in Table 1 below.

[1]Casciano, DA and Gaylor, DW (1983). Statistical criteria for evaluating chemicals as positive or negative in the hepatocytes DNA repair assay. *Mutat Res.* 122:81-86.

Table 1. Summary of UDS Assay Results[a]

Concentration (µg/mL)	Mean Net Nuclear Grains	Cells with ≥5 NNG[b]	Mean Cytoplasmic Grains	Viability Relative to Control (%)	Cells in S-phase/1500 cells (%)
Vehicle Control	-2.35±1.26	6.67	19.94	100.0	0.13
Positive Control	25.40±8.13	94.67	19.09	62.0	0.00
0.25	-2.67±0.99	6.00	19.24	101.4	0.07
0.50	-2.49±1.47	9.33	19.22	95.1	0.20
1.00	-2.09±1.19	9.33	20.33	100.5	0.07
2.50	-1.67±0.64	10.00	19.10	104.1	0.00
5.00	-2.21±1.77	8.00	16.95	92.4	0.07
10.0	-0.75±1.10	13.33	15.19	79.1	0.07

[a]Data were obtained from pp 18-20 of the study report.
[b]NNG = net nuclear grains

The mean net nuclear grain counts of treated cultures were comparable to the vehicle control value. The positive control induced a significant increase in UDS, confirming the sensitivity of the assay.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Based on the reported findings, the test article did not induce UDS in rodent hepatocytes.

B. REVIEWER COMMENTS: The primary cell line used was appropriate and was prepared and maintained with good laboratory techniques. The maximum dose was consistent with the limit dose recommended by harmonized guidelines. Appropriate positive and negative controls were tested. Although the method of statistical analysis was not provided, criteria for statistical evaluation of the results were based on published approaches and considered sufficient. Sufficient number of cells were evaluated for each dosage level. The number of heavily labeled S-phase cells was low and did not interfere with the assay. Our reviewers note that cultures treated with 2.5 and 10 µg/mL had cells with ≥5 net nuclear grain count that exceeded the vehicle historical control range of 0.00-9.30 (10.00 and 13.33, respectively). This response did not meet the criteria for a positive response, however. Overall, the UDS assay was acceptable. **The data indicate that the test article did not induce unscheduled DNA synthesis in cultures of primary rat hepatocytes at the doses tested.**

(7)

C. STUDY DEFICIENCIES:

- The homogeneity, stability, and concentration of the test formulations were not verified.

The protocol deviation was minor and would not adversely affect the outcome and interpretation of the study results.

D. STUDY CLASSIFICATION:

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482 for other genotoxic mutagenicity data.