12-15-03

DATA EVALUATION RECORD

PXTS (Polymeric xylenol tetrasulfide)
MRID 46062617

Bacterial Reverse Mutation Test
OPPTS 870.5100

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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EPA WAM: Killian Swift, Ph.D.

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

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

Signature: _____
Date: 7/11/04

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation *(Bacterial system, Salmonella typhimurium; E. coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

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

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

SYNONYMS: PXTS

CITATION: Stankowski Jr., L.F. (2002) *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with PXTS. Covance Laboratories Inc., Vienna, Virginia. Study Number 23134-0-409OECD. May 13, 2002. MRID 46062617. Unpublished.

SPONSOR: Akzo Nobel Functional Chemicals Inc.
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EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria using the plate-incorporation protocol (MRID 46062617), strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A were exposed to PXTS (100% a.i., Batch# 6, bottle no. 1 and lot 1685-11-1). Concentrations in the main reversion assay were determined by a preliminary toxicity test with TA100 and WP2*uvr*A (6.67-5000 µg/plate, ±S9). *S. typhimurium* strains were incubated with concentrations of 3.33, 10.0, 33.3, 100, 333, and 500 µg/plate in the presence of mammalian metabolic activation (from Sprague-Dawley rats induced with Aroclor 1254; +S9) and 1.00, 3.33, 10.0, 33.3, 50.0, and 66.7 µg/plate in the absence of mammalian metabolic activation (-S9). WP2*uvr*A was exposed to 33.3, 100, 333, 667, 1000, and 2000 µg/plate, +S9, and 10.0, 33.3, 100, 333, 667, and 1000 µg/plate, -S9. Because of a slight increase in revertants at 500 µg/plate in TA100, +S9, the dose range was shifted up in the confirmatory assay for the *Salmonella* strains (10-1000 µg/plate). For the -S9 confirmatory assay, the dose range was shifted down to a range of 0.333-50 µg/plate due to cytotoxicity observed at 50 µg/plate and higher in TA100 in the first assay. The confirmatory assay in WP2*uvr*A, +S9, used a dose range of 100-3330 µg/plate, while that for the -S9 condition used a range of 10-667 µg/plate.

PXTS was tested up to cytotoxic concentrations. Severe decreases in spontaneous revertants were noted in TA100 at ≥33.3 µg/plate and in WP2*uvr*A at ≥667 µg/plate, both -S9. In the initial

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PXTS was tested up to cytotoxic concentrations. Severe decreases in spontaneous revertants were noted in TA100 at ≥33.3 µg/plate and in WP2*uvr*A at ≥667 µg/plate, both -S9. In the initial mutagenicity assay, background lawn inhibition was observed in strains TA98, TA100, and TA1537 at concentrations ≥333 µg/plate (+S9), in TA100 at ≥10.0 µg/plate (-S9), in all *Salmonella* strains at doses ≥33.3 µg/plate (-S9), and in WP2*uvr*A at concentrations of 2000 µg/plate (+S9) and ≥100 µg/plate (-S9). In the confirmatory assay, background growth inhibition was observed at doses of 333 µg/plate, +S9 in TA100 and TA1537, in all *Salmonella* strains at ≥500 µg/plate, and at ≥1000 µg/plate in WP2*uvr*A. Reduced or absent background lawns were noted at ≥3.33 µg/plate (TA100), ≥33.3 µg/plate (all *Salmonella* strains), and ≥100 µg/plate (WP2*uvr*A), -S9. Precipitation of the test article was generally noted at doses of 333 µg/plate or higher in all strains, ±S9. The positive controls induced appropriate responses in each strain. **There was no evidence of induced mutant colonies over background or a concentration-related positive response. Therefore, the test article is considered negative in the bacterial reversion assay.**

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The homogeneity, stability, and concentrations of the test formulations were not verified.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

PXTS

Description: Black solid
Lot/Batch #: Lot No. 1685-11-1/ Batch No. 6, Bottle no. 1
Purity: 100 % a.i.
CAS # of TGAI: Not provided
Structure not available
Solvent Used: Ethanol

2. Control Materials:

Negative: Tester strain and S9 were plated alone as a check of sterility
Solvent (final conc'n): Ethanol (100 µL)
Positive: Nonactivation:

Sodium azide 2.0 µg/plate TA100, TA1535
2-Nitrofluorene 1.0 µg/plate TA98

Other (list):
ICR-191 2.0 µg/plate TA1537
4-Nitroquinoline-N-oxide 1.0 µg/plate WP2*uvr*A

Activation:

2-Aminoanthracene (2-anthramine) 2.5 µg/plate all *S. typhimurium* strains
Other (list):
2-Aminoanthracene (2-anthramine) 25.0 µg/plate WP2 *uvr*A
Benzo[a]pyrene 2.5 µg/plate TA98

3. Activation: S9 derived from

X	induced	X	Aroclor 1254 (200 mg/mL corn oil)	X	Rat (male Sprague-Dawley)	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 mix composition: The S9 homogenate was purchased from Molecular Toxicology, Inc. Two different lots were utilized: Lot 1324 (35.0 mg/mL protein) and Lot 1350 (39.2 mg/mL protein). Fresh S9 mix was prepared prior to each use. The S9 homogenate was mixed with 4 µmol $NADP^+$, 5 µmol glucose-6-phosphate, 3.3 µmol KCl, 8 µmol $MgCl_2$, 100 µmol sodium phosphate buffer (pH 7.4), and water. The final S9 mix contained 10% S9 concentrate.

4. Test organisms: *S. typhimurium* strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538	X	list any others WP2*uvr*A		

Properly maintained? [X] Yes [ ] No

Checked for appropriate genetic markers (*rfa* mutation, R factor)? [X] Yes [ ] No

5. Test compound concentrations used:

Preliminary Cytotoxicity Test
Non-activated conditions: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate
TA100 and WP2*uvr*A
Activated conditions: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate
TA100 and WP2*uvr*A

Main Assay (*S. typhimurium* strains)
Non-activated conditions: 1.00, 3.33, 10.0, 33.3, 50.0, and 66.7 µg/plate
Activated conditions: 3.33, 10.0, 33.3, 100, 333, and 500 µg/plate

Main Assay (WP2*uvr*A)
Non-activated conditions: 33.3, 100, 333, 667, 1000, and 2000 µg/plate
Activated conditions: 10.0, 33.3, 100, 333, 667, and 1000 µg/plate

Confirmatory Assay (*S. typhimurium* strains)
Non-activated conditions: 0.333, 1.00, 3.33, 10, 33.3, and 50.0 µg/plate
Activated conditions: 10.0, 33.3, 100, 333, 500, and 1000 µg/plate

Confirmatory Assay (WP2*uvr*A)
Non-activated conditions: 10.0, 33.3, 100, 333, 500, and 667 µg/plate
Activated conditions: 100, 333, 667, 1000, 2000, and 3330 µg/plate

One plate was prepared for each concentration, strain, and condition for the preliminary toxicity test. Three replicates for each strain, concentration, and condition were counted for the number of revertant colonies in the mutagenicity test. The solvent and positive control plates were assayed in triplicate as well.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay:

X standard plate test
_ pre-incubation (_ minutes)
_ "Prival" modification *(i.e. azo-reduction method)*
_ spot test
_ other

2. Protocol: Dose selection was based on the results of a preliminary toxicity test conducted for the test article in which ten doses were tested (6.7, 10.0, 33.3, 67.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate). In the main assay, all the bacterial tester strains were tested in the absence and presence of metabolic activators for all test concentrations. The plate incorporation method of analysis was used in both the preliminary toxicity test and mutagenicity tests. An aliquot (100

µL) of the bacterial suspension of an overnight culture (0.5×10^9 cells/mL), 100 µL of the solvent or test article or 50 µL of the positive control, and 500 µL of S9 or 500 µL of water was added to 2.0 mL of the selective molten (45±2°C) top agar (0.7% [w/v] agar and 0.5% [w/v] NaCl supplemented with 0.5 mM histidine/biotin (*Salmonella*) or 0.5 mM tryptophan (*E. coli*). This mixture was vortexed and poured onto a petri dish containing 25 mL of minimal agar (Vogel-Bonner medium E supplemented with 1.5 w/v agar and 0.2% glucose) and was allowed to solidify. The cultures were inverted and incubated for 52±4 hours at 37 ± 2°C. Revertant colonies were counted by hand or by automatic colony counter from three replicate plates per strain, concentration, and condition. Plates that were not counted immediately after incubation were stored at 0.1-10°C. Both precipitate and background lawn were evaluated by eye and microscopically.

3. Statistical Analysis: Mean numbers of revertant colonies and their standard deviations were calculated for each strain, concentration, and condition. No statistical analyses were performed. The treatment of the data was considered to be appropriate.

4. Evaluation Criteria: A positive response in TA98, TA100, and WP2*uvr*A was defined as a dose-dependent, ≥2-fold increase in the mean number of revertants per plate relative to the control plates for at least one bacterial strain. A positive response in TA1535 and TA1537 was defined as a dose-dependant, ≥3-fold increase in mean revertants over the control values for at least one bacterial strain.

For the assay to be considered valid, the following criteria must be met: 1) strains TA98, TA100, TA1535, and TA1537 must display a sensitivity to crystal violet; 2) strains TA98 and TA100 must grow in culture broth supplemented with ampicillin (thereby demonstrating pKM101 plasmid presence); 3) at least 3 doses must be evaluated; 4) cell densities $\geq 0.5 \times 10^9$ bacteria/mL must be plated; 5) the positive control (±S9) must produce a at least a 3-fold increase in revertants over the corresponding vehicle control; and 6) all vehicle control cultures must produce a characteristic number of spontaneous revertants per plate within the historical control range (listed below).

Bacterial Strain	Revertant Range
TA98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2*uvr*A	5-40

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: Bacterial strains TA100 and WP2*uvr*A were exposed to 10 doses ranging from 6.67 to 5000 µg/plate, ±S9. Thinning or disappearance of the background lawn was observed in TA100 at concentrations ≥33.3 (-S9) and ≥333 µg/plate (+S9). The background lawn was reduced in WP2*uvr*A strain at doses ≥66.7, -S9, and ≥667

µg/plate, +S9. Precipitation was noted at doses ≥333 (+S9) and ≥100 (-S9) µg/plate in the TA100 plates and ≥667 µg/plate (±S9) in the WP2*uvr*A plates.

B. MUTAGENICITY ASSAY: The results of the initial mutation assay are presented in Table 1 below. Background lawn inhibition was observed in strains TA98, TA100, and TA1537 at concentrations ≥333 µg/plate (+S9), in TA100 at 10.0 µg/plate (-S9), in all *Salmonella* strains at doses ≥33.3 µg/plate (-S9), and in WP2*uvr*A at concentrations ≥100 µg/plate (-S9) and 2000 µg/plate (+S9). Concentrations ≥333 µg/plate (+S9) in TA98 and TA100, ≥333 µg/plate (+S9) in TA1535 and TA1537, and ≥667 µg/plate (±S9) in the WP2*uvr*A strain produced precipitation. The test article did not induce a significant increase in revertants in any strain, ±S9. A dose-dependent increase (approximately 1.6-fold over the control values) was observed in TA100, +S9. This increase was considered by the study author to be a "statistical aberration due to random fluctuation of the spontaneous revertant frequency" and unrelated to the test article. The positive control compounds displayed an appropriate increase in revertants compared to the controls, demonstrating the sensitivity of the assay.

Table 1. Summary of Initial Mutagenicity Assay[a]

	TA98	TA100	TA1535	TA1537	WP2 *uvrA*	
+S9						
Vehicle Control	29±4	101±4	19±8	11±2	Vehicle Control	17±2
3.33 µg/plate	37±6	106±12	23±3	12±2	33.3 µg/plate	23±3
10.0 µg/plate	38±6	104±9	19±3	9±3	100 µg/plate	21±2
33.3 µg/plate	41±6	114±5	19±4	9±4	333 µg/plate	30±6
100 µg/plate	36±1	115±15	19±2	13±3	667 µg/plate	24±2[c]
333 µg/plate	31±8[b, c]	124±14[b, c]	13±3[c]	10±6[b,c]	1000 µg/plate	37±6[c]
500 µg/plate	35±6[b, c]	162±17[b, c]	16±4[c]	7±6[b,c]	2000 µg/plate	18±5[b, c]
Positive Control	384±70	1093±184	146±17	113±13	Positive Control	380±19
-S9						
Vehicle Control	21±5	91±17	16±2	6±2	Vehicle Control	16±3
1.00 µg/plate	25±6	88±15	12±3	8±1	10.0 µg/plate	15±4
3.33 µg/plate	23±6	75±7	10±2	5±2	33.3 µg/plate	15±4
10.0 µg/plate	20±4	71±5[b]	12±4	7±5	100 µg/plate	17±3[b]
33.3 µg/plate	14±5[b]	3±4[b]	5±0[b]	5±2[b]	333 µg/plate	18±8[b]
50.0 µg/plate	15±7[b]	0±0[b]	4±3[b]	2±2[b]	667 µg/plate	1±2[b, c]

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	TA98	TA100	TA1535	TA1537	WP2 *uvrA*	
66.7 µg/plate	8±3[b]	0±0[b]	2±3[b]	1±1[b]	1000 µg/plate	0±0[b, c]
Positive Control	234±29	1131±63	826±18	781±268	Positive Control	220±28

[a] Data obtained from study Tables 4-5 (pp. 24-25).
[b] Reduced background lawn
[c] Precipitate

The results of the confirmatory assay are presented in Table 2 below. Severe decreases in spontaneous revertants, +S9, were noted at ≥333 µg/plate in TA1537, and at the top dose used in all strains, +S9. No revertants were observed in TA100 and TA1537 at 50 µg/plate, -S9, and severe reductions in spontaneous revertants were noted in WP2 *uvrA* at ≥333 µg/plate, -S9. Background lawn inhibition was observed at 333 µg/plate (TA100 and TA1537), 500 µg/plate (TA100, TA1535, and TA1537), 1000 µg/plate (all *Salmonella* strains), and ≥1000 µg/plate (WP2 *uvrA*), +S9. Concentrations ≥3.33µg/plate (TA100), ≥33.3 µg/plate (all *Salmonella* strains) and ≥100 µg/plate (WP2 *uvrA*), -S9, induced either a reduced or absent background lawn. Precipitation was observed at doses ≥500 µg/plate, +S9, in all *Salmonella* strains and at 3330 µg/plate, +S9, or 500 µg/plate, -S9, in WP2 *uvrA*. The test article did not induce a significant increase in revertants in any strain, ±S9.

Both the negative and positive cultures produced expected responses, consistent with historical control ranges in both experiments.

Table 2. Summary of Confirmatory Mutagenicity Assay[a]

	TA98	TA100	TA1535	TA1537	WP2 *uvrA*	
+S9						
Vehicle Control	40±3	101±6	38±11	8±3	Vehicle Control	20±2
10.0 µg/plate	38±5	99±11	34±2	11±4	100 µg/plate	27±1
33.3 µg/plate	33±2	99±18	35±7	8±5	333 µg/plate	31±2
100 µg/plate	39±12	95±5	29±4	13±3	667 µg/plate	28±10
333 µg/plate	39±9	73±13[b]	25±7	1±2[b]	1000 µg/plate	25±9[b]
500 µg/plate	38±7[c]	79±64[b, c]	20±4[b, c]	0±0[b, c]	2000 µg/plate	19±11[b]
1000 µg/plate	28±16[b, c]	0±0[c, d]	8±7[b, c]	0±0[c, d]	3330 µg/plate	0±0[b, c]
Positive Control	389±37	397±35	122±5	93±8	Positive Control	489±94
-S9						
Vehicle Control	26±7	93±8	21±7	8±2	Vehicle Control	15±8

	TA98	TA100	TA1535	TA1537	WP2 *uvrA*	
0.333 µg/plate	19±4	102±5	24±4	6±4	10.0 µg/plate	14±3
1.00 µg/plate	24±3	87±10	16±3	8±1	33.3 µg/plate	18±1
3.33 µg/plate	21±7	99±5[b]	18±4	8±1	100 µg/plate	19±4[b]
10.0 µg/plate	22±4	93±11[b]	23±8	8±5	333 µg/plate	3±3[b]
33.3 µg/plate	14±3[b]	66±34[b]	20±8[b]	7±2[b]	500 µg/plate	0±1[b, c, e]
50.0 µg/plate	11±7[b]	0±0[b]	5±8[b]	0±0[b]	667 µg/plate	0±1[b, c, e]
Positive Control	250±26	1049±70	792±94	1235±103	Positive Control	181±25

[a] Data obtained from study Tables 7-8 (pp. 27-28).
[b] Reduced background lawn
[c] Precipitate
[d] Background lawn absent
[e] Actual mean and standard deviation were 0.33±0.578. The discrepancy in data reporting was due to rounding.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Based on the results of the initial and confirmatory mutagenicity assays, PXTS was considered not mutagenic in the absence and presence of metabolic activation.

B. REVIEWER COMMENTS: In the first reversion assay, a slight dose-dependent increase in mutant colonies was observed in TA100, +S9 at a dose of 500 µg/plate. This response did not meet the stated criteria for a positive response and was not observed in a confirmatory assay using a slightly higher dose range (10.0-1000 vs. 3.33-500 µg/plate). Based on these findings, the observed slight dose-dependent increase in revertants was appropriately concluded by the authors to not be a treatment-related response. Overall, the study was conducted appropriately and reported clearly; therefore, the study sufficiently evaluated the mutagenicity of PXTS.

C. STUDY DEFICIENCIES:

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

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 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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