12-15-03

DATA EVALUATION RECORD

PXTS (polymeric xylenol tetrasulfide)
MRID 46062620

Mammalian Bone Marrow Chromosome Aberration Test
OPPTS 870.5385

Prepared for

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

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Under Subcontract to

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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.

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

Signature:
Date 7/11/04

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in Sprague-Dawley rats; OPPTS 870.5385 [§84-2]; OECD 475.

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

TEST MATERIAL (PURITY): Polymeric Xylenol Tetrasulfide (100% a.i.)

SYNONYMS: PXTS

CITATION: Erexson, GL. (2002) Chromosomal Aberrations *In Vivo* in Rat Bone Marrow Cells with Polymeric Xylenol Tetrasulfide (PXTS). Covance Laboratories Inc., Vienna, VA. Study Number 23134-0-452OECD, January 9, 2002. MRID 46062620. Unpublished.

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

EXECUTIVE SUMMARY: In a bone marrow chromosomal aberration assay (MRID 46062620), six male Sprague-Dawley rats/dose/harvest time were treated once via oral gavage with PXTS (100% a.i., batch # 6, lot # 1685-11-1) in corn oil at doses of 0, 500, 1000, and 2000 mg/kg bw. Test doses were determined from a preliminary toxicity test (0, 500, 1000, and 2000 mg/kg bw administered to male and female rats) in which no difference in susceptibility between the two sexes was observed; therefore, only males were used in the mutagenicity test. Bone marrow cells were harvested at 18 and 42 hours (2000 mg/kg bw only) post-treatment.

Clinical signs of toxicity were irregular breathing in the 1000- and 2000-mg/kg bw groups (1/6 and 1/12, respectively), salivation (2/12), soft feces (4/12), fecal stains (2/12), or blue skin color (6/12) in the 2000-mg/kg bw group. **There was no significant increase in the frequency of both structural and numerical chromosome aberrations in bone marrow after any treatment time. Therefore, the test article was negative in this *in vivo* chromosomal aberration assay.**

This study is classified as **ACCEPTABLE (GUIDELINE)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5385; OECD 475 for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: PXTS (polymeric xylenol tetrasulfide)

Description: Black paste; the test article should be stored at room temperature
Lot/Batch #: Lot # 1685-11-1/Batch # 6, Bottle No. 1
Purity: 100% a.i.
CAS # of TGAI: Not provided
Structure not available
Solvent Used: Corn oil

2. Control Materials:

Negative control (if not vehicle):		**Final Volume:**	**Route:**
Vehicle:	Corn oil	**Final Volume: 10 mL/kg**	**Route: Oral**
Positive control:	Cyclophosphamide	**Final Dose(s): 60 mg/kg**	**Route: Oral**

3. Test animals:

Species: Rats
Strain: Sprague-Dawley Crl:CD® (SD) BR
Age/weight at study initiation: For preliminary toxicity study: 9 weeks/301-356 g (males) and 198-224 g (females)
For main assay: 8 weeks/242-285 g (males only)
Source: Charles River Laboratories, Inc.
No. animals used per dose Preliminary toxicity assay: 3 males/dose; 3 females/dose
Main assay: 6 males/harvest time
Properly Maintained? Yes

4. Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	0, 500, 1000, and 2000 mg/kg	10 mL/kg	Oral Gavage
Main Study:	0, 500, 1000, and 2000 mg/kg		

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test compound, Vehicle control, Positive control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr		24 hr		48 hr		72 hr
Other:	X	18 hr	X	42 hr						

2. Preliminary Toxicity Assay: Three animals/sex/dose were administered 500, 1000, 2000 mg/kg bw of the test article in corn oil via oral gavage. Animals were examined immediately after dosing, approximately 1 hour after dosing, and at least daily for the reminder of the assay for signs of toxicity and/or mortality.

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2. Cytogenetic Assay:

a. Animal observations: Animals were examined immediately after dosing, about 1 hour after dosing, and at least daily for the reminder of the assay for signs of toxicity and/or mortality.

b. Details of bone marrow extraction: Five animals/dose/harvest time received an intraperitoneal injection of colchicine (2 mg/kg) 1.5-2.5 hours prior to sacrifice. Immediately after sacrifice, the tibias were collected and the marrow contents were flushed and collected into Hank's balanced salt solution (HBSS). The marrow contents were centrifuged, resuspended in 0.075 KCl, centrifuged again, and the marrow suspension was resuspended in fixative (methanol:acetic acid [3:1]).

c. Details of slide preparation: Fixed cells were transferred onto glass slides, air-dried, and stained with 5% Giemsa, air-dried again, and cover slipped.

d. Metaphase analysis:
No. of cells examined per dose: 100 cells/dose/harvest time (structural and numerical aberrations) and 1000 cells/dose/harvest time (mitotic index)

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

e. Evaluation criteria: A positive response was a significant dose-dependent increase in structural aberrations for at least one dose level. All final decisions were based on scientific judgement. The mitotic index was evaluated as an indicator of cytotoxicity of the test article. For an assay to be considered valid, the vehicle and positive control must produce acceptable responses within the historical control range (provided on p. 25 of the study report).

f. Statistical analysis: Ranked analyses (nonparametric) for heterogeneity and trend were utilized on all data. Gaps were not included in the aberration analysis. The statistics used were considered sufficient for this assay.

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY: Signs of toxicity, which included soft feces and fecal staining were observed in 2000-mg/kg bw animals on Day 2 and Day 3, respectively. Based on these results, dose levels of 500, 1000, and 2000 mg/kg bw were evaluated in the main assay. Since no differences between the sexes were observed, only males were used in the main assay.

B. CYTOGENETIC ASSAY: No treatment-related signs of toxicity or mortality were noted in 500-mg/kg bw males. Salivation, irregular breathing, soft feces, fecal stain, and blue skin color were observed in 1000- or 2000-mg/kg bw males (Table 1).

Table 1. Clinical Signs of Toxicity[a]

Concentration (mg/kg bw)	Harvest Time (Hours)/ Observation Time	Signs of Toxicity				
		Salivation	Irregular Breathing	Soft Feces	Fecal Stains	Blue Skin Color
500	18					
	immediate	0	0	0	0	0
	1 hour	0	0	0	0	0
	1 day	0	0	0	0	0
	2 days	N/A	N/A	N/A	N/A	N/A
1000	18					
	immediate	0	1	0	0	0
	1 hour	0	0	0	0	0
	1 day	0	0	0	0	0
	2 days	N/A	N/A	N/A	N/A	N/A
2000	18					
	immediate	1	0	0	0	0
	1 hour	0	0	0	0	0
	1 day	0	0	0	0	0
	2 days	N/A	N/A	N/A	N/A	N/A
2000	42					
	immediate	1	1	0	0	0
	1 hour	0	0	0	0	0
	1 day	0	0	0	0	0
	2 days	0	0	4	2	6

[a] Data obtained from pg. 14 of the study report.

Summary data of the chromosome aberration assay are provided below in Table 2. Mitotic indices were comparable across all concentration groups. There were no remarkable increases in either structural chromosome aberrations or numerical chromosome changes induced by treatment. The positive control produced a significant increase in cells with chromosomal aberrations, confirming the sensitivity of the assay.

Table 2. Summary of Chromosome Aberration Assay Results[a]

Concentrations (mg/kg bw)	Harvest Time (Hours)	Average mitotic index (%)	No. of Endoreduplicated cells	No. of Polyploid Cells	Chromosomal Aberrant Cells (%)
Vehicle Control	18	2.00±1.02	0.0±0.00	0.0±0.00	1.2±0.97
	42	5.40±0.76	0.0±0.00	0.0±0.00	0.0±0.00
Positive Control	18	0.28±0.07	0.0±0.00	0.0±0.00	56.1±3.07*
500	18	1.86±0.85	0.0±0.00	0.0±0.00	0.6±0.40
1000	18	0.96±0.76	0.0±0.00	0.0±0.00	2.0±0.71
2000	18	3.80±1.31	0.0±0.00	0.0±0.00	0.4±0.24
	42	4.64±0.36	0.0±0.00	0.0±0.00	0.2±0.20

[a] Data obtained from pg. 21.
* $p \leq 0.05$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Clinical signs of toxicity in the rats included salivation, irregular breathing, soft feces, fecal stains, and blue skin color in 2000-mg/kg treated rats. Irregular breathing was also observed in 1/5 rats at 1000-mg/kg bw, but no clinical signs of toxicity were observed at 500-mg/kg bw. The test article did not induce a significant increase in the percentage of cells with chromosomal aberrations, polyploids, or endoreduplications.

B. REVIEWER COMMENTS: Six male Sprague-Dawley rats were administered the test article in corn oil via oral gavage in single doses of 0, 500, 1000, and 5000 mg/kg and were sacrificed at two time points following dosing: 18 hours (all doses) and 42 hours (control and high dose). The use of males only in the aberration assay was appropriate as there was no sex-specific clinical toxicity noted in the preliminary assay. The selected high dose produced signs of toxicity (clinical signs) as recommended by harmonized guidelines. Marrow extraction and slide preparation and evaluations were conducted in accordance with harmonized guidelines. Criteria for a positive response and a valid assay were provided and were adequate for the study. There were no major deficiencies that would have adversely affected the outcome or interpretation of the results. Overall, the study was conducted appropriately. The test article did not induce chromosomal aberrations when administered to male rats via oral gavage at a dose

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range of 500-2000 mg/kg. Therefore, the test article is negative in this *in vivo* chromosomal aberration assay.

C. STUDY DEFICIENCIES:

- The author stated the light cycle was extended in order to make clinical observations.
- The homogeneity, stability, and concentration of the test formulations were not conducted at the request of the Sponsor.

These protocol deviations were 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 870.5385; OECD 475 for *in vivo* cytogenetic mutagenicity data.