12-15-03

DATA EVALUATION RECORD

Polymeric Xylenol Tetrasulfide (PXTS)
MRID 460626-21

Study Type: Mammalian Erythrocyte Micronucleus Test (Mouse)
OPPTS 870.5395

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

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

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

SYNONYMS: Not provided

CITATION: Erexson, G.L. (2002) *In Vivo* Mouse Micronucleus Assay with Polymeric Xylenol Tetrasulfide (PXTS). Study conducted by Covance Laboratories Inc. (Vienna, Virginia) and submitted under MRID 46062621. Unpublished.

SPONSOR: Akzo Nobel Functional Chemicals, LLC, Dobbs Ferry, New York

EXECUTIVE SUMMARY:

In a Crl:CD-1® (ICR) BR mouse bone marrow micronucleus assay (MRID 460626-21) a preliminary dose range-finding study involved 3 mice/sex/dose exposed once by oral gavage to PXTS dissolved in corn oil (Batch No. 6; Bottle No. 1; Lot No. 1685-11-1, Exp. 9/21/04) at doses of 500, 1000, or 2000 mg/kg bw. Animals were observed for clinical toxicity signs after dosing of test article, 1-hour following, and at least once a day for 48 hours after dosing. The high dose to be utilized in the micronucleus assay was the limit dose of 2000 mg/kg. No differences were observed between sexes and only males were used in subsequent tests.

PXTS dissolved in corn oil was dosed by oral gavage at levels of 500, 1000, and 2000 mg/kg to male Crl:CD-1® (ICR)BR mice. Bone marrow was harvested at 24 hours, 6 animals/dose level. 12 additional animals were sacrificed after 48 hours; 6 animals for vehicle control and 6 for 2000 mg/kg dose level. Observation for signs of clinical toxicity were performed immediately after dosing, within 1 hour following dose, and at least once a day for 48 hours. Positive control article, cyclophosphamide (Sigma, Lot No. 108H0568; CAS No. 6055-19-2) was dissolved in sterile deionized water, administered once by oral gavage, and bone marrow harvested after 24 hours. Only animals dosed with 2000 mg/kg of test article and sacrificed after 48 hours showed signs of clinical toxicity (fecal staining, hypoactive, pale body) and mortality, while vehicle control and all animals that were dosed with 500 and 1000 mg/kg showed no signs of clinical

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toxicity following dosing or 24 and 48 hour harvests.

While there were signs of clinical toxicity, one incident of mortality, and statistically significant decreases in the PCE:NCE ratio 48 hours after the higher dosing level (2000 mg/kg) was administered there was no significant reduction in the percentage of micronucleated PCEs. The positive control showed a statistically significant increase in PCEs at the 24 hour harvest time point. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Polymeric Xylenol Tetrasulfide (PXTS)

Description: Black paste

Batch/Sample #: Batch No. 6; Bottle No. 1

Purity:

CAS # of TGAI: Not reported

Structure: Not reported

Solvent Used: Corn oil (Welsh, Holme, and Clarke); Lot No. 12-394; CAS No. 8001-30-7

2. Control Materials:

		Final Volume:	Route:
Negative control (if not vehicle) :			
Vehicle:	Corn oil	**Final Volume:** 10 mL/kg	**Route:** oral gavage
Positive control :	Cyclophosphamide (monohydrate)	**Final Dose(s):** 80 mg/kg	**Route:** oral gavage

3. Test animals:

Species:	Mouse
Strain:	Crl:CD-1® (ICR) BR strain
Age/weight at study initiation:	Mice were 8 weeks old at the start of treatment; males weighed 30.0-34.4 g and females weighed 21.1-26.9 g at the start of treatment for dose range-finding study. Mice were 9 weeks old at the start of treatment; males weighed 29.3-37.2 g at the start of treatment for micronucleus assay
Source:	Charles River Laboratories, Raleigh, NC
No. animals used per dose	Dose range finding study (3 males; 3 females); Micronucleus assay (6 males; 12 males with 2000 mg/kg dose and vehicle control)
Properly Maintained?	Yes

4. Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	500, 1000, 2000 mg/kg	10 mL/kg	oral gavage
Main Study:	500, 1000, 2000 mg/kg	10 mL/kg	oral gavage

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

Test compound, vehicle, and positive controls:

Dosing:		once								
Sampling (after last dose):		24 hr		48 hr						
Other:										

2. Tissues and Cells Examined:

Bone marrow:	hind limb bones (tibia)
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	not reported
Other (if other cell types examined, describe): erythrocytes (to determine ratio of PCE:NCE)	first 500

3. Details of slide preparation: The hind limb bones (tibia) were removed from animals and marrow was flushed from the first five surviving animals per group. The bone marrow was transferred to individual centrifuge tubes and combined with 3-5 mL of fetal bovine serum. The cells were centrifuged, spread on slides, and air-dried. Slides were coded prior to analysis, fixed in methanol (for 30 minutes), stained with a combination of May- Gruenwald and Giemsa stain and coverslips were permanently mounted. Slides were scored for the presence of micronuclei (MN), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) was determined.

4. Evaluation Criteria: A positive result was defined as a statistically significant increase in the number of micronucleated PCEs for at least one of the dosing levels and a statistically significant dose-related response. A negative result was defined as any deviation from the above criterion. In addition to statistical analysis, the final evaluation included biological relevance consideration.

5. Statistical methods: Statistical data analysis of untransformed proportions of cells with micronuclei/animal and untransformed ratios of PCE:NCE was performed using analysis of variance (ANOVA) for homogeneous variances and ranked proportions for heterogeneous variances. Statistically significant ANOVA ($p \leq 0.05$) involved further analysis using Dunnett's t-test to determine significant difference between dose groups and vehicle control. Statistical analysis was performed at both 24 and 48 hour sampling time points. Additionally, positive control data were compared with vehicle control data and evaluated at the 1% ($p \leq 0.01$) level. Our reviewers consider the analyses used to be appropriate.

II. REPORTED RESULTS

A. ANALYTICAL ANALYSIS: Not reported.

B. PRELIMINARY TOXICITY ASSAY: Three mice/sex were exposed once to the test material via oral gavage at concentrations of 500, 1000, or 2000 mg/kg body weight and sacrificed 48 hours after treatment. Animals were observed for clinical signs of toxicity (fecal staining, hypoactivity, pale body) and mortality. Clinical signs of toxicity were observed after 48 hours in 1 male at the 2000 mg/kg dose level with signs of a rough haircoat, fecal staining, and slight hypoactivity. All other animals appeared normal and healthy at all dose levels. Differences in toxicity according to sex were not noted; therefore, the following tests were performed with male mice only.

C. MICRONUCLEUS ASSAY: Male mice in groups of 6 per dose or vehicle were harvested 24 hours after dosing (exceptions: 6 additional animals for dose level 2000 mg/kg and 6 animals for vehicle control 48 hours after dosing). Treatments at dose levels of 500 and 1000 mg/kg and controls, both vehicle and positive, showed no signs of clinical toxicity or mortality. However, the 48 hour time point of the 2000 mg/kg dose resulted in 1 mortality, 2 animals with fecal staining, and 1 animal with fecal staining, hypoactivity, and pale body. Table 1 presents the percentage of incidences of micronuclei in the different treatment groups. No significant increases in micronucleated PCEs were noted in any of the test article treatments. There was a statistically significant decrease in the PCE:NCE ratio at the 2000 mg/kg dose level 48 hours after treatment; however, the test article was considered negative for the mouse micronucleus assay. The positive control showed significant increases in percentage of micronucleated PCEs exhibiting a positive response to the assay.

TABLE 1. MICRONUCLEUS SUMMARY DATA[a]

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000/ANIMAL ± S.E.	RATIO PCE:NCE MEAN ± S.E.
CONTROLS				
Vehicle	Corn Oil	24 hr	0.0 ± 0.01	0.58 ± 0.05
		48 hr	0.05 ± 0.02	0.55 ± 0.03
Positive	CP 80 mg/kg	24 hr	1.75 ± 0.17*	0.47 ± 0.06
TEST ARTICLE	500 mg/kg	24 hr	0.02 ± 0.01	0.47 ± 0.04
	1000 mg/kg	24 hr	0.05 ± 0.02	0.46 ± 0.08
	2000 mg/kg	24 hr	0.07 ± 0.03	0.56 ± 0.08
		48 hr	0.08 ± 0.02	0.38 ± 0.06**

[a] Data obtained from page 17 in the study report.
CP= Cyclophosphamide
PCE= Polychromatic erythrocyte
NCE= Normochromatic erythrocyte
* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.

Tables 2 (24 hr harvest time point) and 3 (48 hr harvest time point) show the number of micronucleated PCEs/2000 PCEs per animal and treatment doses and the ratios of PCE:NCE for the male mouse micronucleus assay.

TABLE 2. MICRONUCLEUS TEST-24 HOUR HARVEST INDIVIDUAL MALE DATA*

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 2000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	MALE			
VEHICLE CONTROL	Corn Oil	8346	1	0.54
		8347	0	0.39
		8357	0	0.67
		8358	0	0.69
		8366	0	0.60
POSITIVE CONTROL	CP 80 mg/kg	8345	47	0.46
		8348	35	0.34
		8356	34	0.68
		8361	26	0.43
		8369	33	0.41
TEST ARTICLE	500 mg/kg	8334	1	0.59
		8338	0	0.41
		8343	1	0.46
		8351	0	0.54
		8360	0	0.37
	1000 mg/kg	8350	2	0.56
		8354	0	0.54
		8355	1	0.43
		8359	1	0.58
		8367	1	0.17
	2000 mg/kg	8336	1	0.25
		8342	2	0.61
		8349	3	0.72
		8352	0	0.56
		8652	1	0.68

* Data obtained from page 18 in the study report.
CP= Cyclophosphamide
PCE= Polychromatic erythrocyte
# MN PCEs= Micronucleated PCEs
NCE= Normochromatic erythrocyte

TABLE 3. MICRONUCLEUS TEST-48 HOUR HARVEST INDIVIDUAL MALE DATA[a]

TREATMENT		ANIMAL NUMBER	# MN PCE'S/2000 PCES	RATIO PCE:NCE
48 HOUR HARVEST	MALE			
VEHICLE CONTROL	Corn Oil	8339 8344 8364 8371 8373	0 2 0 1 2	0.52 0.48 0.59 0.50 0.64
TEST ARTICLE	2000 mg/kg	8335 8340 8341 8362 8363	2 2 2 0 2	0.19 0.28 0.46 0.45 0.52

[a] Data obtained from page 19 in the study report.
PCE= Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: PXTS did not induce a significant increase in micronuclei in bone marrow and the compound was evaluated as negative in the mouse micronucleus assay under the conditions of this assay. Although, PXTS did induce signs of clinical toxicity and mortality and significantly decreased PCE:NCE ratios in the 2000 mg/kg animals 48 hours after dosing.

B. REVIEWER COMMENTS: Although at the 48 hour harvest time point, the dose level of 2000 mg/kg induced toxicity and mortality in animals and exhibited a significant decrease in the ratio of PCE:NCE, PXTS did not induce micronuclei in Crl:CD-1® (ICR) BR male mice under these study conditions.

C. STUDY DEFICIENCIES: No major deficiencies were identified in the study; however, minor deviations from guidelines were noted. 1)Purity of test article not reported. 2)Animal housing temperature ranged from 64 to 79 °F (17.7-26.1 °C) when guidelines specify 19-25 °C.

D. STUDY CLASSIFICATION: This study is classified as **Acceptable-Guideline** and meets the guideline requirements for an *in vivo* mammalian cytogenetics - erythrocyte micronucleus assay in mice (OPPTS 870.5395 [§84-2]).