1-12-04

DATA EVALUATION REPORT

Polymeric Xylenol Tetrasulfide (PXTS)
MRID 46062614

Study Type: 90-Day Oral Toxicity in Rodents
OPPTS 870.3100

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	*Nikki Maples, M.S.*	Date 1/12/04
Independent Reviewer	*Brenda Tondi, Ph.D.*	Date 1/7/04
ICF Program Manager	*Kara B. Altshuler, Ph.D.*	Date 1/9/04
Versar Program Manager	*Linda Phillips, Ph.D.*	Date 1/12/04

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.

EPA Reviewer: Tim McMahon, Ph.D.
Senior Toxicologist, Antimicrobials Division

Signature:
Date 7/1/04

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [gavage]-[rats]; OPPTS 870.3100 [§82-1] (rodent); OECD 408.

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

TEST MATERIAL (PURITY): PXTS (>99% a.i.)

SYNONYMS: None reported

CITATION: Findlay, J. (2003) 90-Day Oral Toxicity Study of PXTS in Rats. Experimur, Chicago, IL. Laboratory Project ID 02-178, May 7, 2003. MRID 46062614. Unpublished.

SPONSOR: Akzo Nobel Functional Chemicals LLC, 5 Livingston Ave, Dobbs Ferry, NY.

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 46062614) PXTS (>99% a.i., Lot # 1685-23) was administered to 10 Sprague-Dawley rats/sex/dose by gavage at dose levels of 0, 50, 200, and 500 mg/kg/day in Tricaprylin (5 days/week). An additional 10 rats/sex were administered 0 or 500 mg/kg/day for the 90-day treatment period and evaluated after a 4-week recovery period. The concentration formulations were analyzed and found to be 99.6-104.2% of the theoretical concentration.

No treatment-related effects were observed in mortality (one control male died due to pyelonephritis), ophthalmology, and neurologic parameters. Treatment-related clinical signs of toxicity included cyanosis and discolored inguinal fur were observed in the 500-mg/kg/day group. Findings consistent with hemolysis were observed in a dose-dependent manner with reductions in RBC counts, hemoglobin, hematocrit, and MCHC and increases in MCV, MCH, and absolute and relative reticulocyte counts, and total bilirubin occurring at doses ≥200mg/kg/day. Significant decreases in hemoglobin were observed in the 50-mg/kg/day females as well. The hemolytic nature of the test article was also responsible for pigmentation observed in several organs (lungs, spleen, liver, and kidneys), by promoting the aggregation of heme iron from red blood cells into hemosiderin (observed microscopically as coarse, brown cytoplasmic granules). Body weight and weight gain, which correlated with reduced food consumption, were significantly reduced in the 500-mg/kg/day treated rats. The decreased food consumption; however, was not of sufficient magnitude to be solely responsible for the significant reductions in body weight observed at this dose level. These effects were also

(2)

observed at the 200-mg/kg/day treatment level, but with less severity. Elevated absolute and relative spleen organ weights in the 200- and 500-mg/kg/day treated animals correlated with the gross observation of enlarged spleens and microscopic observations of passive congestion in the red pulp and extramedullary hematopoiesis. Males and females at 50 mg/kg/day had increased relative spleen weight, but the increase was only significant in the females. Several other organs demonstrated increased relative weights in the 500-mg/kg/day group which correlated with lower body weights; therefore, these increases were secondary to the weight loss and not a direct toxicological effect. Lesions consistent with irritation caused by contact with the test article were observed during microscopic examination of the lungs, bronchi, nose, and stomach. These inflammatory responses were attributed to aspiration of micro-droplets of the dose formulations during the gavage procedures and were observed at all treatment levels.

The reversibility of the systemic and topical irritative effects following a 4-week recovery period was confirmed with the following observations: absence ofcyanosis and inguinal fur staining; increase in body weight gain; elevation of RBC counts, hemoglobin, hematocrit, MCHC, MCV, and MCH with a decrease in the absolute and relative reticulocyte counts and total bilirubin. The increased relative spleen weights, pigmentation ofthe spleen, liver, kidneys, and lungs, and the irritative effects observed in the nose, lungs, bronchi, and stomach were lessened, but these effects were not fully reversed after the 4-week recovery period. It was concluded that PXTS treatment for 90 days at these dose levels did not cause irreversible target organ toxicity.

In the 90-day toxicity study (MRID 46062614), the systemic LOAEL was originally stated to be 50 mg/kg/day (lowest dose tested), based on increased relative spleen weight in females, decreased hemoglobin in females, and histopathology of the lungs, spleen, stomach, and kidneys of both sexes. However, review of the data by the Antimicrobials Division Toxicology Endpoint Selection Committee (ADTC) indicates that the effects occurring at the 50 mg/kg/day dose are not significant enough to warrant a LOAEL at this dose. Therefore, the ADTC determined that the 50 mg/kg/day dose be considered the NOAEL for this study, and the 200 mg/kg/day dose level the LOAEL, based on the same effects stated for the 50 mg/kg/day dose level. **Thus, the LOAEL is 200 mg/kg/day, based on increased relative splenic weight (female), decreased hemoglobin (female), and histopathology of the lungs, spleen, stomach and kidneys (both sexes). The NOAEL is determined to be 50 mg/kg/day.**

This 90-day oral toxicity study in the rat **ACCEPTABLE-GUIDELINE**; and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: PXTS

Description:	Black tar-like material
Lot/Batch #:	Lot # 1685-23
Purity:	>99% a.i. (Purity data not provided in study report but taken from MRID 46062616 based on the same test article lot number)
Compound Stability:	The stability of the neat test article was not provided, and was reported to be the responsibility of the Sponsor.
CAS # of TGAI:	Not reported

2. Vehicle and/or positive control: Tricaprylin, Lot # 71K1667 (>99% a.i. as reported in MRID 46062615) and 22K1209 (>99% pure as reported in MRID 46062614)

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley [Crl:CD®(SD)IGS BR]	
Age/weight at study initiation:	7-8 weeks/males ranged between 222-285 g and females ranged between 159-200 g	
Source:	Charles River Laboratories, Portage, MI	
Housing:	Single-housed in suspended stainless steel cages	
Diet:	Harlan Teklad Certified Rodent Diet #8728C, *ad libitum* (All feed lots were analyzed for chemical or biological contaminants; no known contaminants were reported present.)	
Water:	City of Chicago municipal tap water, *ad libitum* (No known contaminants were present in the water that may interfere with the outcome of the study.)	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air Changes:	Not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	2 weeks	

B. STUDY DESIGN:

1. In life dates - Start: June 12, 2002 End: October 22, 2002

2. Animal assignment: Animals were assigned randomly, based on body weight, to the test groups noted in Table 1. Body weight variation did not exceed ±20% of the mean weight for each sex. The animals were dosed 5 days per week over 90 days for a total of 64 doses. The recovery animals were observed for 28 days after the termination of treatment.

TABLE 1: Study design

Test Group	Dose to Animal (mg/kg/day)	Treatment Volume (mL/kg)	Core Group		Recovery Group	
			# Male	# Female	# Male	# Female
1	0	2	10	10	10	10
2	50	2	10	10	-	-
3	200	2	10	10	-	-
4	500	2	10	10	10	10

3. Dose selection rationale: The dose levels were selected by the Sponsor based on the results of other toxicity studies with the test article.

4. Dose preparation and analysis: Dose formulations were prepared by mixing appropriate amounts of test article heated to 70°C with Tricaprylin at 2.5, 10, and 25% (w/v) concentrations for the 50, 200 and 500 mg/kg dose groups, respectively. Four dose formulations were prepared throughout the study (June 25, July 24, August 14, and September 8, 2002). Concentration and homogeneity analyses were performed on the day of dose preparation. Homogeneity analyses were performed by taking two samples from three sampling levels in the preparation container of the low- and high-dose formulations while concentration analyses were performed on two samples from the mid-dose formulations. The stability of the test article in the vehicle at dose formulations of 25 and 500 mg/mL was analyzed by the testing facility prior to the commencement of the study. The dose formulations were found to be stable for at least 28 days at room temperature.

Results -

Homogeneity Analysis: The analyses for homogeneity resulted in 97.6-106.8% and 99.2-100.6% of the target concentrations of the low (2.5%) and high (25.00%) concentrations, respectively.

Concentration Analysis: The samples analyzed were 99.6-104.2% of the target concentration of the mid-dose concentration (10.00%).

The analytical data indicated that the mixing procedure was adequate with minimal deviation between dose formulation strata, and that the variance between nominal and actual dosage to the animals was acceptable (within 10%).

5. Statistics - Levene's test was used to analyze continuous data. A one-way analysis of variance (ANOVA) was used to analyze homogenous data ($p>0.001$). Dunnett's two-tailed t-test was used to analyze significant effects as compared to the vehicle control and data were considered significant at $p\leq0.05$. Automated motor activity data were analyzed with ANOVA (one-way, multivariate and or repeated measures). Data were manually entered into Excel®

where the mean and standard deviation were calculated; statistical analyses were performed with Systat® 10. Statistical analyses were not performed on macroscopic or histopathology observations. The statistical analyses were appropriate for this assay. Additional analyses would have been useful in interpreting some of the data, such as a chi-square test and a test for trends such as the Cochran-Armitage, Jonckheere, or Wilcoxon Rank Sum test.

C. METHODS:

1. Observations:

1a. Cageside Observations: Animals were inspected at least once daily for moribundity and mortality.

1b. Clinical Examinations: Clinical observations were conducted daily during quarantine, prior to study initiation, and weekly throughout the study.

1c. Neurological Evaluations: The following evaluations were performed blind across all groups during Week 12: a functional observational battery (F.O.B.) with assessment of gait in an open field arena, grip strength, righting reflex, extensor thrust, response to tail pinch, gross evaluation of auditory response, and visual and tactile placing; and motor activity assessment in an open field for 20-minutes by an automated motor activity system.

2. Body weight: Animals were weighed at randomization and weekly during the study. Fasted body weights were collected prior to necropsy .

3. Food consumption and compound intake: Food consumption for each animal was determined on the same day each week as body weight measurements.

4. Ophthalmoscopic examination: Eyes were examined by indirect funduscope during the quarantine period, Week 13, and at the end of the recovery period in all rats, as appropriate.

5. Hematology & Clinical Chemistry: Blood was collected from the abdominal aorta for hematology and clinical chemistry from all surviving animals. Animals were fasted prior to blood collection. The CHECKED (X) parameters were examined. It was reported that a negative reading for gamma glutamyl transferase values is recorded as either a 0 or <3 in the raw data, considered to be 0, and reported as 0 in the summary data.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Automated red cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphate	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine kinase		Serum protein electrophoresis
X	Lactate dehydrogenase (LDH)	X	A/G ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. Urinalysis: Urinalysis was not performed.

7. Sacrifice and Pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination. Rats surviving to scheduled sacrifice were fasted over night and euthanized with an overdose of sodium pentobarbital. The CHECKED (X) tissues were collected for histological examination in the control and 500-mg/kg/day animals. In addition, the kidneys, liver, lungs with bronchi, nose, spleen, and stomach were evaluated in all other treatment groups and recovery animals, and all gross lesions were examined microscopically. The (XX) organs, in addition, were weighed. All tissues were fixed in 10% neutral buffered formalin except the testes, which were fixed in Bouin's solution. Tissues were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow (sternum and femur)*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes (mandibular and mesenteric)*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	Harderian Gland
	Gall bladder (not rat)*	X	Epididymides*+		OTHER
	Bile duct (rat)	X	Prostate*	X	Bone (sternum and femur)
X	Pancreas*	X	Seminal vesicles*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries (with oviducts)*+	X	Skin*
X	Trachea*	X	Uterus (with cervix)*+	X	All gross lesions and masses*
X	Lung*	X	Mammary gland*	X	Sciatic nerve
X	Nose*	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity - Selected clinical observations are summarized in Table 2. Cyanosis was observed in 16/20 males and 9/20 females in the 500-mg/kg/day treatment group, and in one out of the ten 200-mg/kg/day females. Cyanosis persisted for one week after cessation of treatment in one 500-mg/kg/day recovery female, but was absent by the end of the 4-week recovery period. Other clinical signs observed in 500-mg/kg/day rats included discolored inguinal fur (brown/yellow), red material around the nose, discoloration around the mouth, discolored paws, salivation, irritability and noisy breathing. With the exception of the discolored inguinal fur, these signs were observed with low incidence. Alopecia, particularly of the forelimbs, was frequently observed in both control and treatment groups.

Table 2. Summary of selected clinical observations[a]

	Incidences* during Treatment Period								Incidences* during Recovery Period			
	Male				Female				Male		Female	
(mg/kg/day)	0	50	200	500	0	50	200	500	0	500	0	500
# Examined	20	10	10	20	20	10	10	20	10	10	10	10
Alopecia (forelimbs)	5	4	1	8	3	3	3	8	2	3	1	5
Alopecia (abdomen)	2	-	-	2	-	-	1	4	1	1	-	1
Cyanosis	-	-	-	16	-	-	1	9	-	-	-	1
Discolored inguinal fur (yellow and/ or brown)	-	-	-	10	-	-	-	6	-	-	-	-

[a] Data obtained from Table 2 of the study report (pp. 24 and 25).
* Incidences indicate the observation was recorded at least once during the study.

2. Mortality - One control male died on Day 91 with pathology consistent with pyelonephritis (multiple white foci in the kidney). Additional observations in the decedent included enlarged prostate caused by an abscess, mild multifocal hemorrhages on the mandibular lymph node, moderate multifocal myodegeneration of the heart with minimal infiltration of mononuclear cells, minimal multifocal mineralization of the tunica media of the aorta, marked diffuse cystitis of the urinary bladder, and minimal diffuse hypercellularity of the bone marrow in both the femur and sternum. There were no treatment-related deaths during the study period.

3. Neurological Evaluations - No treatment-related effects were seen in the FOB or motor activity tests.

B. BODY WEIGHT AND WEIGHT GAIN: Average body weight and body weight gain for selected weeks during treatment and during the 4-week recovery period are indicated in Tables 3 and 4, respectively. Mean body weight was statistically-significantly decreased in the 500-mg/kg/day rats throughout the 13-week treatment period, as compared to controls. Mean body weight was also reduced in 200-mg/kg/day males during Weeks 2-13 and in 200-mg/kg/day females during Weeks 6-13, but these reductions did not reach statistical significance. Significant weekly reductions in body weight gains were noted in 500-mg/kg/day males (Weeks 1, 4 and 12) and females (Weeks 1 and 6), with total body weight gain significantly reduced in both; total body weight gain was 65% of controls in males and 70% of controls in females. Significantly reduced body weight gain was also observed in 200-mg/kg/day males at Week 5, with total body weight gain being 83% and 82% of controls in 200-mg/kg/day males

and females, respectively.

Mean body weights were significantly lower than controls in the 500-mg/kg/day males throughout the 4-week recovery period, but only at Week 14 in the 500-mg/kg/day females. Weekly body weight gains in the 500-mg/kg/day groups, as indicated in Table 5, were significantly higher throughout the 4-week recovery period in the males, and at Week 15 in females, as compared to recovery controls. Total body weight gain during the recovery period was also significantly elevated in the high-dose animals; males were 305% of controls and females were 211% of controls.

TABLE 3. Selected average body weights and body weight gains during treatment[a]

Dose (mg/kg/day)	Body Weights (g±S.D.)				Total Weight Gain	
	Week 0	Week 1	Week 7	Week 13	g	% of control
Male						
0	246±14.7	298±22.9	454±49.7	508±71.5	262±61.9	100
50	249±18.1	303±28.1	440±59.4	509±75.5	260±60.1	99
200	245±14.1	296±21.9	411±57.7	463±72.2	218±62.8	83
500	244±12.4	276±19.5*	384±40.7*	414±37.0*	171±29.9*	65
Female						
0	178±9.9	198±12.8	272±21.8	293±23.0	115±16.4	100
50	181±10.9	200±10.6	265±20.6	284±18.5	103±13.3	90
200	184±11.4	200±14.8	265±26.6	277±23.1	94±14.8*	82
500	177±7.9	189±8.4	244±15.0*	257±24.7*	81±20.1*	70

[a] Data obtained from Tables 5-8 (pp 32-33; 35-36; 39; 42) in the study report.
* Statistically different ($p \leq 0.05$) from the control.

TABLE 4. Average body weights and body weight gains during recovery[a]

Dose (mg/kg/day)	Body Weights (g±S.D.)				Total Weight Gain	
	Week 14	Week 15	Week 16	Week 17	g	% of control
Male						
0	537±59.9	549±60.8	558±62.3	549±61.7	22±11.5	100
500	441±40.4*	465±43.3*	486±43.7*	483±44.0*	67±11.7*	305
Female						

Dose (mg/kg/day)	Body Weights (g±S.D.)				Total Weight Gain	
	Week 14	Week 15	Week 16	Week 17	g	% of control
0	301±22.2	304±23.0	308±24.2	304±22.3	9±4.7	100
500	278±20.0*	285±21.2	289±23.7	288±22.7	19±11.2*	211

[a] Data obtained from Tables 5-8 (pp. 34, 37, 40, and 43) in the study report.
* Statistically different ($p \leq 0.05$) from the control.

Table 5. Weekly body weight gain during 4-week recovery[a]

Dose (mg/kg/day)	Body Weight Gains (g±S.D.)			
	Week 14	Week 15	Week 16	Week 17
Male				
0	10±6.9	12±4.7	9±8.8	-10±5.7
500	26±6.1*	24±4.6*	20±4.7*	-3±3.2
Female				
0	6±4.8	3±4.1	4±6.8	-4±7.6
500	10±5.0	7±3.6*	5±5.6	-2±3.4

[a] Data calculated from Tables 7 and 9 (pp. 40 and 43) in the study report.

C. FOOD CONSUMPTION:

1. Food consumption - Average weekly food consumption for selected weeks during the treatment and recovery periods are indicated in Table 6. Weekly food consumption was generally lower than vehicle controls in the 500-mg/kg/day treatment groups, but not always significantly. It was significantly decreased in 500-mg/kg/day treated rats in Weeks 1 (12%) and 4 (10%) in males, and in Weeks 1 (10%) and 2 (10%) in females. There was a slight, nonsignificant decrease in weekly food consumption in 200-mg/kg/day males as compared to controls. It is notable that the decrease in food consumption does not appear to be of magnitude large enough to be totally responsible for the reductions observed in body weight in these treated animals.

During the recovery phase, weekly food consumption in the high-dose animals was slightly higher than the controls, with a significant increase (17%) observed at Week 14 in the females.

47

Table 6. Selected average weekly food consumption (g/week)[a]

Mean Weekly Food Consumption (±S.D.)								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**20**	**10**	**10**	**20**	**20**	**10**	**10**	**20**
Week 1	177±22.1	182±26.6	176±12.6	155±22.1*	129±9.2	130±11.6	127±10.1	116±7.8*
2	183±25.8	180±29.6	176±13.1	169±27.0	133±9.3	134±13.9	134±13.7	120±13.9*
4	182±21.5	180±25.1	176±21.4	163±23.3*	136±12.1	137±14.0	137±12.1	131±14.4
8	181±23.3	181±31.8	171±23.5	166±30.9	128±10.7	127±11.9	122±15.2	122±14.3
13	145±32.7	157±29.7	155±24.2	158±32.2	118±10.2	113±9.9	118±8.4	117±18.0
14[b]	187±19.8	-	-	203±20.4	118±8.6	-	-	138±27.3*
17[b]	210±20.3	-	-	212±17.4	154±7.5	-	-	156±14.9

[a] Data obtained from Tables 3 and 4 (pp. 26-31) in the study report.
[b] Indicates recovery group animals (10/group).
* Significantly different from the control ($p \leq 0.05$).

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were noted; one 200-mg/kg/day male exhibited a focal equatorial cataract in the left eye.

E. BLOOD ANALYSES:

1. Hematology - Treatment-related dose-dependent hemolytic effects were observed in the 200- and 500-mg/kg/day treatment groups as indicated in Table 7. Statistically-significant reductions in RBC count, hemoglobin, hematocrit, and MCHC were observed in ≥200-mg/kg/day males and females, and in hemoglobin of the 50-mg/kg/day females. Statistically-significant increases were observed in MCV, MCH, and percent and absolute number of reticulocytes in the ≥200-mg/kg/day males and females. Percent eosinophils were significantly decreased in the ≥200-mg/kg/day males and in 500-mg/kg/day females, with a significant decrease in the absolute eosinophils in the 500-mg/kg/day males. The high-dose groups' red cell morphology exhibited an increase in variation of cell size and hemoglobin concentration variance. Hyperchromia was found in both control and treatment groups with similar incidences; however, the response was enhanced in the 500-mg/kg/day males. Additionally, RBC morphology changes were observed in the 500-mg/kg/day males and females and included anisocytosis and hemoglobin concentration variance.

Following recovery, many of the observed effects during treatment were reversed in the high-dose group. Compared to recovery controls, significant increases were observed in HGB, HCT, MCV, and MCH in both males and females, and a decrease of MCHC in females. Significant decreases in the percent and absolute reticulocytes were observed in the 500-mg/kg/day males and females. After recovery, platelets were significantly reduced in the 500-mg/kg/day females,

but this value is within the range of values for this age and strain of the species ($1120\pm220\times10^3/\mu L$, Jain, 1986). The significant changes observed in eosinophils during treatment were not observed after the 4-week recovery period; however, absolute and percent basophils were significantly increased in the 500-mg/kg/day males, and percent basophils in the 500-mg/kg/day females, as compared to recovery controls.

Table 7. Selected hematology summary results[a]

Parameter Group Mean (± S.D.)								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Treatment Period								
RBC ($x10^6$ cells/µL)	8.82±0.23	8.72±0.34	7.28±0.40*	6.02±0.53*	8.21±0.23	7.90±0.35	6.58±0.35*	5.40±0.72*
HGB (g/dL)	15.5±0.4	15.3±0.4	13.8±0.7*	12.4±0.8*	15.5±0.3	14.8±0.5*	13.1±0.6*	11.7±1.4*
HCT (%)	45.3±1.3	44.6±1.7	41.0±2.0*	38.1±2.5*	43.7±0.9	42.0±1.3	38.1±1.8*	36.2±4.8*
MCHC (g/dL)	34±0.5	34±0.5	34±0.3*	32±0.6*	36±0.4	35±0.5	34±0.4*	32±0.7*
MCV (fL)	51.3±2.5	51.2±1.1	56.3±1.7*	63.5±3.0*	53.2±1.7	53.2±1.7	58.0±1.8*	67.0±2.2*
MCH (pg)	17.6±0.8	17.6±0.4	19.0±0.6*	20.6±0.7*	19.0±0.5	18.8±0.5	19.9±0.5*	21.8±0.9*
Retic (%)	1.7±0.3	2.0±0.4	5.4±0.6*	12.8±4.0*	1.9±0.3	2.0±0.4	6.8±1.5*	18.1±4.7*
Retic ($x10^9$ cells/L)	153.6±22.9	176.1±32.2	393.6±52.2*	761.4±214.1*	154.4±21.5	154.7±25.7	447.4±91.9*	971.4±273.4*
Eos (%)	1.7±0.5	1.3±0.4	0.9±0.5*	0.7±0.3*	1.6±0.7	1.5±0.8	1.1±0.4	0.6±0.3*
Eos ($x10^6$ cells/µL)	0.11±0.052	0.10±0.043	0.07±0.034	0.06±0.026*	0.07±0.025	0.07±0.044	0.05±0.018	0.03±0.014
Hyperchromia incidence (severity)[b]	6	7	1	5 (++) 5 (+++)	1 (+) 7 (++) 2 (+++)	4 (+) 4 (++) 2 (+++)	4 (+) 2 (++)	2 (+) 5 (++) 3 (+++)
HGB conc. variance (severity)[b]	0	0	0	10 (+++)	0	0	0	1 (+) 4 (++) 4 (+++)
Anisocytosis (severity)[b]	0	0	0	4 2 (++) 1 (+++)	0	0	0	3 (+) 2 (++)

(14)

Parameter Group Mean (± S.D.)								
Sex	Male				Female			
mg/kg/day	0	50	200	500	0	50	200	500
n	9	10	10	10	10	10	10	10
Recovery Period								
n	10			10	10			10
RBC ($x10^6$ cells/µL)	9.04±0.336			8.92±0.348	8.02±0.356			8.26±0.292
HGB (g/dL)	15.6±0.7			16.8±0.4*	15.0±0.5			16.6±0.5*
HCT (%)	46.0±1.9			49.4±0.9*	43.0±1.3			48.9±1.4*
MCHC (g/dL)	34±0.5			34±0.5	35±0.4			34±0.4*
MCV (fL)	50.9±1.9			55.4±1.7*	53.7±2.3			59.3±1.3*
MCH (pg)	17.2±0.7			18.8±0.6*	18.7±0.8			20.2±0.4*
Retic (%)	1.9±0.5			0.8±0.2*	1.9±0.3			1.0±0.2*
Retic ($x10^9$ cells/L)	167.6±38.7			71.5±18.5*	155.5±25.2			81.9±12.6*
PLAT ($x10^3$ cells/µL)	1065±124			1009±125	1281±136			1154±132*
Bas (%)	0.28±0.10			0.41±0.09*	0.25±0.11			0.38±0.10*
Bas ($x10^6$ cells/µL)	0.02±0.009			0.03±0.014*	0.02±0.007			0.02±0.012
Hyperchromia incidence (severity)[b]	8 1 (++)			5	7 1 (++) 2 (+++)			2 1 (++)
HGB conc. variance (severity)[b]	1			0	0			0
Anisocytosis (severity)[b]	0			0	0			0

[a] Data obtained from Tables 17-28 (pp. 72-87) in the study report.

[b] Severity classifications were not defined in the study report. Our reviewers assumed that the + symbol denoted an enhanced response.
* Statistically different ($p \leq 0.05$) from the control.

2. Clinical Chemistry - Several clinical chemistry parameters were significantly changed, as compared to controls, during the treatment or recovery period as indicated in Table 8. Total bilirubin levels were significantly increased in ≥200-mg/kg/day males and females. Cholesterol was significantly reduced in ≥200-mg/kg/day females; a nonsignificant decrease was also observed in the ≥200-mg/kg/day males. Other statistically significant decreases were observed in triglyceride and potassium levels in the 500-mg/kg/day males.

Following the 4-week recovery, creatinine and ALT levels were significantly decreased in high-dose males; A/G ratios and total bilirubin were decreased and sodium was increased in high-dose females. Cholesterol values in the 500-mg/kg/day females displayed a similar decrease that was observed at terminal sacrifice but the value was not significant.

Table 8. Selected clinical chemistry summary results[a]

Parameter Group Mean (± S.D.)								
Sex	Male				Female			
mg/kg/day	0	50	200	500	0	50	200	500
n	9	10	10	10	10	10	10	10
Treatment Period								
CHOL (mg/dL)	48.0±11.4	51.5±17.6	42.5±7.4	35.1±6.8	57.0±9.9	53.2±7.2	44.9±3.8*	47.8±10.1*
T. BIL (mg/dL)	0.10±0.00	0.14±0.05	0.22±0.04*	0.30±0.08*	0.18±0.04	0.18±0.04	0.24±0.05*	0.40±0.07*
K (mmol/L)	4.6±0.2	4.7±0.1	4.6±0.2	4.3±0.3*	4.2±0.2	4.2±0.3	4.0±0.2	4.2±0.8
TRIG (mg/dL)	41.7±13.7	41.7±13.4	33.8±13.1	27.8±9.5*	28.9±9.2	27.5±7.3	23.9±6.9	24.6±10.4
Recovery Period								
n	10			10	10			10
CHOL (mg/dL)	49.5±20.2			43.5±10.2	67.1±17.3			60.8±12.1
CREA (mg/dL)	0.4±0.0			0.3±0.0*	0.4±0.0			0.4±0.1
ALT (U/L)	33.3±6.7			25.9±5.0*	30.0±9.3			32.1±19.6
A/G Ratio	1.0±0.1			1.0±0.1	1.1±0.1			1.0±0.0*
T. BIL (mg/dL)	0.12±0.04			0.10±0.00	0.19±0.03			0.12±0.04*
Na (mmol/L)	142±1			143±1	141±1			142±1*

[a] Data obtained from Tables 13-16 (pp. 60-71) in the study report.

* Statistically different ($p \leq 0.05$) from the control.

F. SACRIFICE AND PATHOLOGY:

1. Organ weight - Treatment-related changes in organ weights were observed in the spleen as indicated in Table 9. Other organ weight changes are not included in Table 9 because they were not considered to be a direct toxicological effect, but rather a reflection of the decreased body weight observed in the high-dose treated animals. Statistically significant increases in absolute and relative spleen organ weights were observed in 200- and 500-mg/kg/day males and females, and in 50-mg/kg/day females, as compared to controls. Significantly increased absolute and relative splenic weights in the high-dose treatment groups persisted after the 4-week recovery period, when compared to recovery controls.

The high-dose group also exhibited increased organ-to-body weight ratios in the brain, heart, kidneys, and liver. Following the 4-week recovery period, relative brain (males), heart (males and females), kidneys (males), and liver (males) organ weights remained elevated, as well as relative adrenal weight in the females. These changes in relative organ weights were correlated to the decreased body weight. Significant absolute organ weight changes observed in 500-mg/kg/day treated rats after recovery included decreased testes (males) and increased heart weight (females).

Table 9. Average absolute and relative (to-body) weight spleen values[a]

Group Mean (± S.D.)								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Treatment Period								
Fasted Body Weight (g)	488 ±57.9	488 ±72.8	442 ±69.4	390 ±34.4*	278 ±25.1	271 ±19.5	265 ±23.3	230 ±25.4*
Spleen (g)	0.764 ±0.143	0.877 ±0.213	1.436 ±0.267*	2.667 ±0.656*	0.534 ±0.043	0.581 ±0.066	0.881 ±0.102*	1.961 ±0.776*
Relative Spleen (%)	0.157 ±0.020	0.179 ±0.025	0.325 ±0.039*	0.680 ±0.132*	0.193 ±0.018	0.214 ±0.021*	0.336 ±0.053*	0.839 ±0.288*
Recovery Period								
n	**10**			**10**	**10**			**10**
Fasted Body Weight (g)	524 ±60.4			459 ±43.2*	289 ±21.9			271 ±21.7
Spleen (g)	0.842 ±0.128			1.049 ±0.195*	0.563 ±0.064			0.767 ±0.135*
Relative Spleen (%)	0.161 ±0.019			0.227 ±0.028*	0.198 ±0.027			0.283 ±0.042*

[a] Data obtained from Tables 29-32 (pp. 88-91) in the study report.
* Significantly different from the control ($p \leq 0.05$).

2. Gross pathology - Treatment-related incidences of enlarged spleens and lung pigmentation observed in 200- and 500-mg/kg/day treated rats are indicated in Table 10. The incidence of enlarged spleens was reduced after the 4-week recovery period in both 500-mg/kg/day males and females; lung pigmentation was absent after recovery.

Table 10. Incidences of selected macroscopic findings after treatment[a]

Group Mean (± S.D.)								
Sex	Male				Female			
mg/kg/day	0	50	200	500	0	50	200	500
n	9	10	10	10	10	10	10	10
Treatment Period								
Enlarged spleen	0	0	5	10	0	0	1	10
Lung pigmentation	0	0	0	2	0	0	0	0
Recovery Period								
n	10			10	10			10
Enlarged spleen	1			2	0			3
Lung pigmentation	0			0	0			0

[a] Data obtained from Table 37 (p. 96) in the study report.

3. Microscopic pathology - Treatment-related microscopic changes were observed in the lungs, bronchi, nose, spleen, stomach, liver, and kidneys and are presented in Tables 11a and 11b. The lungs, bronchi, and nose all displayed signs of irritation with inflamation, exudate, and ulceration that increased in incidence and severity with increasing dose. The stomach exhibited increasing incidence and severity of nonglandular epithelial hyperplasia and nonglandular hyperkeratosis. Passive congestion in the red pulp of spleens correlated with the increased splenic weights and size observed in the 200- and 500-mg/kg/day treated animals. Extramedullary hematopoiesis was observed in the red pulp of the spleen in all test groups; however, the incidence and severity increased in a dose-dependent manner in the treated groups. Pigmentation was observed in the spleen, liver, kidneys, and lungs which was likely due to metabolism of sulfur from the test article resulting in hemosiderin deposition in these organs.

After 4 weeks of recovery, the effects observed in the respiratory tract were mostly reversible, with minimal or mild irritation of the lungs persisting in no more than 2 of the high-dose animals, and no incidence of nose irritation or injury. There was also a decrease in the severity of the stomach histopathology during the recovery period. In addition, hemosiderin deposition was partially reversed during recovery.

Table 11a. Incidences of selected histopathology findings after treatment[a]

Microscopic Observations								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Lungs								
Subacute inflammation								
minimal	3	3	4	4	2	1	3	3
mild	1	5	6	1	0	1	4	1
moderate	0	2	0	2	0	0	0	2
marked	0	0	0	1	0	0	0	0
Bronchioles interlumenal exudate								
minimal	0	0	2	1	0	0	1	1
mild	0	0	2	2	0	0	0	0
moderate	0	0	0	1	0	0	0	2
marked	0	0	0	1	0	0	0	0
Bronchioles epithelial hyperplasia								
minimal	0	3	1	1	0	0	1	0
mild	0	1	4	3	0	0	3	5
moderate	0	0	1	1	0	0	0	0
marked	0	0	0	1	0	0	0	0
Bronchiolitis obliterans								
minimal	0	0	0	1	0	0	2	0
mild	0	0	4	3	0	0	0	2
moderate	0	0	0	0	0	0	0	1
Macrophage pigmentation								
minimal	0	2	3	5	0	2	4	1
mild	0	0	5	2	0	0	1	1
moderate	0	0	2	0	0	0	0	0
Nose								
Lumen purulent exudate								

Microscopic Observations								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
minimal	0	0	0	0	0	0	1	0
mild	0	0	1	1	0	0	1	0
moderate	0	0	0	2	0	0	0	0
Ventral meatus ulceration								
mild	0	0	0	0	0	0	1	0
moderate	0	0	0	1	0	0	0	0
marked	0	0	0	1	0	0	0	0
Dorsal meatus ulceration								
minimal	0	0	0	0	0	0	1	0
mild	0	0	1	0	0	0	0	0
marked	0	0	0	1	0	0	0	0
Spleen								
Red pulp passive congestion								
minimal	0	1	2	0	0	0	4	3
mild	0	0	4	3	0	0	4	4
moderate	0	0	4	6	0	0	2	2
marked	0	0	0	1	0	0	0	1
Red pulp pigmentation								
minimal	3	4	0	2	7	7	1	4
mild	0	2	2	5	0	2	1	5
moderate	0	0	8	5	0	1	7	0
marked	0	0	0	0	0	0	0	1
Extramedullary hematopoiesis								
minimal	4	6	0	0	3	2	0	0
mild	0	2	2	5	0	0	4	2
moderate	0	0	8	5	0	0	5	7
marked	0	0	0	0	0	0	1	1

Microscopic Observations								
Sex	**Male**				**Female**			
mg/kg/day	**0**	**50**	**200**	**500**	**0**	**50**	**200**	**500**
n	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Stomach								
Nonglandular epithelial hyperplasia								
minimal	0	1	2	0	1	1	1	3
mild	0	0	3	3	0	7	3	1
moderate	0	0	4	2	0	0	5	7
marked	0	0	1	5	0	0	0	3
Nonglandular hyperkeratosis								
minimal	0	1	2	1	0	3	5	1
mild	0	0	4	3	1	1	0	4
moderate	0	0	3	5	0	0	1	3
marked	0	0	1	1	0	0	0	1
Liver								
Kupffer cells pigmentation								
minimal	0	0	6	7	0	0	7	8
mild	0	0	3	2	0	0	2	2
Kidneys								
Tubular epithelium pigmentation								
minimal	0	0	1	4	0	0	0	3
mild	0	0	0	0	0	0	0	2
Mononuclear cells infiltration								
minimal	0	0	1	5	0	1	0	2

[a] Data obtained from Appendix 21 of the study report (pp. 254-259).

Table 11b. Incidences of selected histopathology findings after recovery[a]

Microscopic Observations				
Sex	**Male (n=10/group)**		**Female (n=10/group)**	
mg/kg/day	**0**	**500**	**0**	**500**
Lungs				

Microscopic Observations				
Sex	**Male (n=10/group)**		**Female (n=10/group)**	
mg/kg/day	**0**	**500**	**0**	**500**
Subacute inflammation				
minimal	0	1	0	1
Macrophage pigmentation				
minimal	0	2	0	1
mild	0	0	0	2
Spleen				
Red pulp passive congestion				
minimal	0	4	0	2
mild	0	1	0	2
Red pulp pigmentation				
minimal	5	1	5	0
mild	3	3	4	0
moderate	0	6	0	3
marked	0	0	0	7
Extramedullary hematopoiesis				
minimal	1	2	3	0
mild	0	0	1	1
Stomach				
Nonglandular epithelial hyperplasia				
minimal	0	4	1	1
mild	0	4	0	6
moderate	0	1	0	0
Nonglandular hyperkeratosis				
minimal	0	4	2	3
mild	0	4	0	6
moderate	0	1	0	1
Liver				
Kupffer cells pigmentation				

Microscopic Observations				
Sex	Male (n=10/group)		Female (n=10/group)	
mg/kg/day	0	500	0	500
minimal	0	5	0	4
mild	0	3	0	5
moderate	0	0	0	1
Kidneys				
Tubular epithelium pigmentation				
minimal	0	1	0	5
mild	0	0	0	2

[a] Data obtained from Appendix 21 of the study report (pp. 260-263).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: PXTS was administered via oral gavage to 10 Sprague Dawley rats/sex/dose at dose levels of 0, 50, 200, and 500 mg/kg/day in Tricaprylin for 5 days/week for 90 days. An additional 10 rats/sex were administered 0 or 500 mg/kg/day for the 90-day treatment period and evaluated after a 4-week recovery period.

No treatment-related effects were observed in mortality, ophthalmology, and neurologicparameters. Body weight and weight gain were reduced in the 500-mg/kg/day treated rats, which correlated with reduced food consumption. These effects were also observed at the 200-mg/kg/day treatment level, but with less severity. Treatment-related clinical signs of toxicity observed in 500-mg/kg/day treated rats included cyanosis and discolored inguinal fur. Cyanosis was concluded to be caused by the presence of hydrogen sulfide, produced by the conversion of free sulfur from the test article by intestinal bacteria. In addition to cyanosis, hydrogen sulfide also causes hemolysis. This is consistent with the hemolytic changes observed in the hematology parameters, notably reductions in RBC counts, hemoglobin, hematocrit, and MCHC and increases in MCV, MCH, and absolute and relative reticulocyte counts at doses ≥200 mg/kg/day. Elevated total bilirubin, observed at doses ≥200 mg/kg/day, were also indicative of erythrocyte destruction caused by the test article. The hemolytic nature of the test article was also responsible for the pigmentation observed in several organs (lungs, spleen, liver, and kidneys), with hemolysis promoting the aggregation of heme iron from the red blood cells into hemosiderin (observed microscopically as coarse, brown cytoplasmic granules). Elevated absolute and relative spleen organ weights in the 200- and 500-mg/kg/day treated animals correlated with both the gross observation of enlarged spleens and the microscopic observations of passive congestion in the red pulp and extramedullary hematopoiesis. Other increases in relative organ weights observed in the 500-mg/kg/day treated animals are concluded to be indicative of the lower body weights in these animals, and not a direct toxicity effect. Additional microscopic observations were results of persistent topical exposure to the test article resulting in signs of irritation in the lungs,

bronchi, nose and stomach. The topical exposure was due to the aerosolization and aspiration of micro-droplets of the dose formulations during the gavage procedures and was observed at all treatment levels.

The reversibility of the systemic and topical effects was confirmed with the absence of cyanosis and the increase in body weight gain during the recovery period in the 500-mg/kg/day animals. Hematology parameters associated with hemopoeisis were elevated during the recovery period as a response to the hemolytic effects of the test article, and support that the hematopoietic system was not damaged by treatment. The topical contact changes observed in the lungs, bronchi, and nose were partially reversed during recovery, as was the pigmentation observed in the spleen, liver, kidneys, and lungs, and increased spleen weights. It was concluded that PXTS treatment for 90 days at these dose levels did not cause irreversible target organ toxicity. Based on the results, the no-observed-adverse-effect-level (NOAEL) was determined to be less than 50 mg/kg/day.

B. REVIEWER COMMENTS: Our reviewers agree with the author that oral treatment with PXTS results in hemolytic effects at dose levels of 200- and 500-mg/kg/day which include reduced RBC, hemoglobin, hematocrit, and MCHC; increased MCV, MCH, and percent and absolute number of reticulocytes; red blood cell morphology with increased variation of cell size and hemoglobin concentration; hyperchromia; elevated total serum bilirubin levels; cyanosis; increased splenic weight and size with congestion and extramedullary hematopoiesis; and pigmentation associated with hemosiderin production in the lungs, spleen, liver, and kidneys. Our reviewers also agree that the lesions observed in the lungs, bronchi, nose, and stomach were contact topical effects due to inhalation of the micro-droplets during the gavage procedure.

Evaluation of 500-mg/kg/day treated animals after a 4-week recovery period indicated that the systemic and topical effects were at least partially reversible. The study utilized doses that were sufficient to cause toxic effects and identify target organs.

In the 90-day toxicity study (MRID 46062614), the systemic LOAEL was originally stated to be 50 mg/kg/day (lowest dose tested), based on increased relative spleen weight in females, decreased hemoglobin in females, and histopathology of the lungs, spleen, stomach, and kidneys of both sexes. However, review of the data by the Antimicrobials Division Toxicology Endpoint Selection Committee (ADTC) indicates that the effects occurring at the 50 mg/kg/day dose are not significant enough to warrant a LOAEL at this dose. Therefore, the ADTC determined that the 50 mg/kg/day dose be considered the NOAEL for this study, and the 200 mg/kg/day dose level the LOAEL, based on the same effects stated for the 50 mg/kg/day dose level. **Thus, the LOAEL is 200 mg/kg/day, based on increased relative splenic weight (female), decreased hemoglobin (female), and histopathology of the lungs, spleen, stomach and kidneys (both sexes). The NOAEL is determined to be 50 mg/kg/day.**

This 90-day oral toxicity study in the rat **ACCEPTABLE-GUIDELINE**; and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

C. STUDY DEFICIENCIES: Minor deficiencies in this study included the absence of dose-trend statistics, omission of blood clotting measurements and organ weights of the epididymides, uterus, and thymus. These minor deficiencies did not alter the outcome of the study.

D. STUDY CLASSIFICATION: This 90-day oral toxicity study in the rat is ACCEPTABLE-GUIDELINE; andsatisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

E. REFERENCES: Jain NC. Schalm's Veterinary Hematology. 4th ed. Philadelphia: Lea & Febinger. 1986.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
	46062614	subchronic	rat	90 days	oral	gavage	50-500	0, 50, 200, 500	<50	50	Hematopoietic system (spleen), body weight	Toxicity