1-12-04

DATA EVALUATION RECORD

Polyxylenol tetrasulfide (PXTS)
MRID 46062616

A developmental toxicity study of orally administered PXTS in rabbits
OPPTS 870.3700

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700 [§83-3b]; OECD 414.

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

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

SYNONYMS: PXTS

CITATION: Faqi, A.S. (2003) A developmental toxicity study of orally administered PXTS in rabbits. IIT Research Institute (IITRI) Chicago, IL. Laboratory project ID 1636 SN6, February 2003. MRID 46062616. Unpublished.

SPONSOR: Akzo Nobel Functional Chemicals LLC
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EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 46062616), polyxylenol tetrasulfide or PXTS (100% a.i., lot/batch # 1685-23) was administered to at least 30 mated female New Zealand White rabbits/dose by gavage at dose levels of 0, 1, 5, 20, or 62.5 mg/kg bw/day from days 6 through 18 of gestation.

Maternal toxicity was observed in all study groups, including the control, and consisted of mortality, clinical signs of toxicity (cyanosis, diarrhea, scant feces, no feces, and red urine), body weight loss, premature delivery, and gross morphological changes (molted lungs and pale liver). Additional gross morphological alterations related to treatment include red/brown pigmentation in the large intestine, fluid in the thoracic cavity, and various types of pigmentation in the lungs, liver, and large intestine; however, these changes did not occur in the control group. Maternal deaths consisted of four control dams, six 1-mg/kg/day dose dams, four 5-mg/kg/day dams, seven 20-mg/kg/day dams, and eighteen 62.5-mg/kg/day dams. Premature delivery occurred in seven control dams, eight 1-mg/kg/day dams, eight 5-mg/kg/day dams, ten 20-mg/kg/day dams, and six 62.5-mg/kg/day dams. Based on similar results that were observed in previous studies that attempted to characterize the developmental toxicity of PXTS in rabbits (Ralph Freudenthal, personal communication), it is apparent that the vehicle, tricaprylin, shows toxicity to rabbits in

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the absence of any chemical treatment. That the apparent tricaprylin maternal toxicity observed in this rabbit study appears to be species-specific is supported by repeated oral dosing studies conducted in rats using the same vehicle (MRID's 46062614; 46062615) in which rats were dosed with up to 500 mg/kg/day of PXTS in tricaprylin with no deaths occurring. Additionally, the reproductive/developmental toxicity screen conducted in rats showed no adverse effects on reproduction at doses up to 500 mg/kg/day, supporting the lack of developmental or reproductive toxicity of PXTS. Dams in the present study administered 62.5 mg/kg/day exhibited a decreased sex ratio and an increased preimplantation loss; these alterations did not reach statistical significance. High mortality in all groups, especially the 62.5-mg/kg/day group, resulted in a small sample size of litters/fetuses. The decreased sex ratio is likely to be an artefact of the small number of litters at the top dose. For preimplantation loss, the lack of a clear dose-response relationship, as well as the small sample size, again limit the assessment of this effect. **The maternal LOAEL is 1 mg/kg/day; a maternal NOAEL cannot be determined because adverse effects occurred at all dose levels.**

A significant increase in the incidence of kidney dilation was observed in fetuses at 62.5 mg/kg/day. Without histopathology data on this organ, however, it is not possible to determine if the dilation was caused by kidney or ureter obstruction, or by another effect. Further, in the absence of historical data, it is not possible to determine the frequency of this endpoint in laboratory controls. According to guidelines, at least 20 litters are needed to make proper comparisons. In this study there were only 13 control litters, twelve 1-mg/kg/day litters, fourteen 5-mg/kg/day litters, nine 20-mg/kg/day /litters, and five 62.5-mg/kg/day litters. **Consequently, the developmental NOAEL is 20 mg/kg/day and the LOAEL is 62.5 mg/kg/day based on kidney dilation, which is conservatively considered an adverse effect.**

This developmental toxicity study in the rabbit is classified as **Unacceptable-guideline** and does not satisfy the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits. Maternal toxicity was evident in all study groups, including the control. The vehicle used (tricaprylin) likely was a contributing factor to the observed maternal effects. Nevertheless, the high level of maternal mortality and premature delivery in all study groups resulted in an insufficient number of dams and litters for analysis.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Polyxylenol tetrasulfide (PXTS)

Description:	A solid material with the consistency of road tar
Lot/Batch #:	1685-23
Purity:	100% a.i.
Compound Stability:	Stable at room temperature
CAS #of TGAI:	Not provided
	The structure is provided in Appendix B (page 51) of the study report.

2. Vehicle and/or positive control: The vehicle was tricaprylin ($C_{27}H_{50}O_6$; Lot # 022K1209; purity >99% a.i.), a clear, colorless to light yellow liquid.

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at study initiation:	Females: 5 months old upon receipt/random sample (20 rabbits) weighed 2.95-4.38 kg the day after receipt Males: 9 months old upon receipt/random sample (5 rabbits) weighed 3.27-3.47 kg the day after receipt	
Source:	Covance Research Products, Inc. (Kalamazoo, MI)	
Housing:	Individually-housed (except during mating) in suspended, stainless-steel cages with automatic watering systems and excrement pans lined with disposable poly pads	
Diet:	During the quarantine period, animals received 150 g/day of either 50:50 mixture of Certified High Fiber Rabbit Diet and Certified Rabbit Diet (#5325 and 5322, respectively, PMI Nutrition International, Inc., Brentwood, MO) or 100% of the high fiber diet. Sperm-positive females received the 50:50 mixture *ad libitum* for the rest of the study. The diet reportedly did not contain levels of contaminants that would interfere with the study.	
Water:	Tap water (City of Chicago) was provided *ad libitum*. Water reportedly did not contain levels of contaminants that would interfere with the study.	
Environmental conditions:	**Temperature:**	22-26 °C
	Humidity:	42-77 %
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	13 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: July 15, 2002 End: August 23, 2002

2. Mating: Mating was conducted over an eight-day period using a staggered-start design to ensure adequate evaluation of developmental toxicity in the fetuses. Approximately 25-50% of each study group was mated on approximately 2 to 4 consecutive workdays. One female and one male rabbit were housed together until mating was observed, for a period of not longer than one hour. Males were of the same strain and from the same source. Mating was confirmed via

sperm-positive vaginal imprints. The day that a sperm-positive vaginal imprint was obtained was designated as gestation day (gd) 0.

3. **Animal Assignment:** Sperm-positive females were assigned to dose groups using a computerized randomization procedure based on body weight as indicated in Table 1. Each animal was identified by a number written on its inner ear with a nontoxic, permanent marker.

TABLE 1: Animal Assignment

Dose (mg/kg bw/day)	0	1	5	20	62.5
# Sperm-Positive Females	32	33	33	33	33

4. **Dose selection rationale:** According to the study protocol, the dose levels were selected on the basis of a previous range-finding study (IITRI Project Number 1636SN1). No further details are provided.

5. **Dosage preparation and analysis:** Dosing formulations at concentrations of 0.3, 1.7, 6.7, and 20.8 mg/mL (to deliver 1, 5, 20, and 62.5 mg/kg) were prepared weekly by mixing appropriate amounts of heated test substance (approximately 90°C) with tricaprylin. Dilution to final volume was performed after the dosing formulations were cooled and stirred; dosing formulations were then placed in amber glass jars and stored at room temperature until use. Samples (~2 mL) were collected from all four preparations to evaluate achieved concentration. To evaluate homogeneity, replicate samples taken from the top, middle, and bottom stratus of the high- and low-dose levels and replicate samples from the mid-dose level were analyzed on 7/18/02 and 7/25/02; these samples were prepared on the same day as analysis. Stability was evaluated on 7/18/02 (Day 0), 7/25/02 (Day 7), 7/29/02 (Day 11), and 8/1/02 (Day 14) from replicate samples taken from the top, middle, and bottom stratus of the high and low doses that were prepared on Day 0.

Results

Homogeneity Analysis: The low dose was determined to be homogeneous with a relative standard deviation (R.S.D.) of 3%, and the high dose was determined to be homogenous with an R.S.D. of 1%.

Stability Analysis: For the low dose, the stability ranged from 97-100%; for the high dose, the stability ranged from 100-104%.

Concentration Analysis: The mean concentrations of all doses prepared for the first and second weeks of dosing were 101-107% and 104-110%, respectively, of the target concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gavage, on gd 6 through 18, at a volume of 3.0 mL/kg of body weight/day. The study report dose not state whether dosing was based on the most recently determined body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: The animals were checked for mortality and moribundity at least once daily during quarantine and gd 0 to 5. During treatment, animals were observed at least twice daily, at least 4 hours apart, for mortality. Clinical observations were recorded once daily 0-3 hours after dosing or once daily on non-dosing days. Full physical examinations were conducted on day 0 and at study termination (gd 29). Body weights were recorded on the day after receipt, at randomization, and on gd 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 29. Food consumption was recorded on the same gestational days as body weights. Surviving animals were sacrificed and necropsied on gd 29; moribund animals were sacrificed *in extremis* and necropsied, and animals that died spontaneously were removed and necropsied.

Examinations at sacrifice consisted of a gross necropsy, cesarean section, and tissue collection. Tissue masses and suspected lesions were dissected, sliced into appropriate-sized sections, and fixed in 10% buffered formalin. The uterus from each female was removed, trimmed of excess fat, and weighed prior to the removal of the fetuses from the uterine horns. The uterus was then weighed with the ovaries, and the number of corpora lutea was recorded for the right and left ovaries. The uterine horns were examined for tissue resorptions and fetal deaths. Resorptions/deaths were counted, recorded, and classified as early resorption (placenta only), late resorption (placenta and fetal remains), early death (fetus weighing <10 grams), or late death (fetus weighing >10 grams). Livers also were removed and weighed.

2. Fetal Evaluations: All viable fetuses were removed, counted, identified in a systematic fashion, weighed, sexed, and examined for any gross abnormalities. The gross external morphological examination consisted of the following: evaluation of body and head shape; sizing and extension of limbs; counting of separate digits; and inspection of skin, umbilicus region, anus, genitals, nares, pinna, eyes, and the oral cavity.

All live fetuses received a wet visceral examination of all recommended structures by a modification of the Staples' method and were sexed internally for verification purposes. Cephalic examinations were conducted on a random collection of live fetuses from each litter. The selected fetuses were decapitated, and each head was fixed in Bouin's solution for a minimum of 48 hours prior to an examination using a modified Wilson's Razor Blade Technique. A stereomicroscope was used to examine all protocol-specified structures. Abnormalities were recorded, and fetal heads were stored in 70% ethanol. All fetuses received a skeletal examination; skeletal examinations of decapitated fetuses were limited to their torsos. Fetuses were skinned, fixed in ethyl alcohol, stained first with Alcian Blue and then with Alizarin Red- S/potassium hydroxide solution, and cleared of excess stain using a clearing

solution. Guideline-recommended bones were counted and examined with a stereomicroscope. Skeletal abnormalities were recorded, and fetal skeletons were preserved in glycerin.

D. DATA ANALYSIS

1. **Statistical analyses:** Systat software (Systat Software Inc., Richmond, CA, version 5.0) was used to perform all statistical comparisons (minimum significance level of $p \leq 0.05$). The body weights of the dams and fetuses were compared by analysis of variance, followed, where appropriate, by the *post-hoc* Dunnett's test. Gross, visceral, and skeletal data were analyzed by Chi-Square/Fisher exact tests (fetal N) or a log-linear model (nesting pups within dam) when the incidence in the treated rabbits was higher than control rabbits.

2. **Indices:** The preimplantaion loss was calculated from cesarean section records of animals in the study using the following formula:

- % preimplantation loss = (total corpora lutea - total implants)/total corpora lutea X 100

3. **Historical control data:** Historical control data are not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations:** Select mortality and clinical observation data are presented in Table 2. Maternal toxicity was evident in all dose groups, including the control group. Thirty-nine animals died prior to scheduled sacrifice, including four control dams, six 1-mg/kg/day dose dams, four 5-mg/kg/day dams, seven 20-mg/kg/day dams, and eighteen 62.5-mg/kg/day dams. The number of deaths in the control group was comparable to the 1-, 5-, and 20-mg/kg/day dose groups, indicating that the vehicle (tricaprylin) had toxic effects on the rabbits.

Clinical observations related to the test substance or to the vehicle include cyanosis, diarrhea, scant feces, no feces, and red urine. Other spontaneous clinical signs that are not considered treatment-related include salivation, discoloration around the mouth, clear nasal discharge, alopecia, cold to touch, vaginal discharge, bloody diarrhea, and brown mucus discharge.

TABLE 2: Mortality and select clinical observations [a]

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Number observed	32	33	33	33	33
Normal	25	28	28	30	30
Animals found dead	4	6	4	7	18
Cyanosis	1	2	1	3	2
Diarrhea	5	4	8	9	5
Scant feces	23	24	27	27	26
No feces	15	12	14	20	13
Red urine	1	2	1	3	2

a Data obtained from page 21 in the study report.

2. Body Weight: There were no statistically significant changes in mean body weights in any treatment group throughout the study. Body weight gain data are summarized in Table 3. The only statistically significant finding is an increase in body weight gain during gd 9-12 in the 5-mg/kg/day dose group compared to the control. There were no significant differences between treatment groups and the control with respect to corrected body weight and corrected body weight gain. Nevertheless, all groups, including the control, lost weight during all treatment period intervals (gd 6-9, 9-12, 12-15, and 15-18), which resulted in an overall corrected body weight loss. Because these weight losses were comparable in treatment and control groups and were limited to the treatment period, they are most likely an effect of the vehicle.

TABLE 3: Mean (±SD) Maternal Body Weight Gain (kg) [a]

Interval	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Pretreatment					
Days 0-3	0.03±0.118	0.05±0.067	0.07±0.074	0.06±0.086	0.07±0.070
Days 3-6	0.02±0.058	0.02±0.069	0.01±0.075	0.01±0.077	0.02±0.060
Treatment					
Days 6-9	-0.15±0.092	-0.16±0.067	-0.15±0.075	-0.19±0.057	-0.19±0.076
Days 9-12	-0.14±0.071	-0.10±0.072	-0.09*±0.071	-0.10±0.071	-0.13±0.065
Days 12-15	-0.09±0.076	-0.11±0.067	-0.11±0.087	-0.14±0.067	-0.13±0.049
Days 15-18	-0.08±0.071	-0.10±0.066	-0.08±0.067	-0.13±0.051	-0.13±0.087

Interval	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Posttreatment					
Days 18-21	0.01±0.171	0.02±0.124	-0.04±0.123	0.02±0.108	-0.10±0.124
Days 21-24	0.07±0.155	0.06±0.186	0.11±0.189	0.07±0.207	0.00±0.142
Days 24-27	0.16±0.143	0.07±0.135	0.12±0.155	0.16±0.12	0.13±0.237
Days 27-29	0.09±0.087	0.07±0.078	0.017±0.058	0.06±0.088	0.15±0.147
Total Gain	0.05±0.349	0.03±0.269	0.04±0.247	-0.01±0.147	-0.28±0.458
Corrected BW Gain	-0.35±0.263	-0.32±0.191	-0.32±0.231	-0.26±0.246	-0.57±0.377

a Data obtained from pages 23 and 25 the study report.
* Statistically different ($p < 0.05$) from the control.

3. Food Consumption: There were no statistically significant changes in mean food consumption in the female rabbits at any time during the study.

4. Gross Pathology: Necropsy observations include mottled and red pigmentation in the lungs, a pale liver, red/brown pigmentation in the large intestine, fluid in the thoracic cavity, and various types of pigmentation in the lungs, liver, and large intestine. Other sporadic findings include red urine, a cyst or tan pus in the uterine horn, a cyst in the fallopian tubes, a subcutaneous abscess in the skin, hair and a white, mucousy substance in the stomach, and a ruptured stomach. There were no treatment-related effects observed in mean liver or uterine weights. Select gross necropsy findings are presented in Table 4.

Table 4: Select gross necropsy observations [a]

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Number examined	25	28	28	30	29
No gross lesions	21	19	22	18	11
Lungs					
- pigmentation, mottled	2	4	1	5	9
- pigmentation, red	0	0	2	0	5
Liver					
- pale	1	3	1	1	5
Large intestine					
- pigmentation, red/brown	0	0	0	0	2
Thoracic cavity					
- fluid	0	2	1	1	3

a Data obtained from pages 16 and 26-27 in the study report.

5. Cesarean Section Data: Thirty-nine dams delivered prematurely (seven control dams, eight 1-mg/kg/day dams, eight 5-mg/kg/day dams, ten 20-mg/kg/day dams, and six 62.5-mg/kg/day dams). This finding is a further indication of vehicle toxicity since the number of premature deliveries in the control group was comparable to the treated groups. There were no test-substance related effects on group mean number of corpora lutea, implantations, live or dead fetuses, resorptions, mean fetal body weight, sex ratio, or preimplantation loss. Cesarean section data are summarized in Table 5.

TABLE 5: Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
# Animals Assigned (Mated)	32	33	33	33	33
# Animals Pregnant	24	26	26	28	29
Pregnancy Rate (%) [b]	75.0	78.8	78.8	84.8	87.9
# Nonpregnant	7	5	5	3	3
Maternal Wastage					
# Died	4	6	4	7	18
# Died Pregnant	NA	NA	NA	NA	NA
# Died Nonpregnant	NA	NA	NA	NA	NA
# Aborted	NA	NA	NA	NA	NA
# Premature Delivery	7	8	8	10	6
Total # Corpora Lutea	142	144	159	123	65
(Corpora Lutea/Dam)	10±1.2	10± 2.9	10±2.9	9±1.9	11±1.9
Total # Implantations	122	98	132	80	45
(Implantations/Dam)	9±1.3	7±3.6	8±3.7	6±4.0	8±1.4
Total # Litters	13	12	14	9	5
Total # Live Fetuses	103	77	105	61	32
(Live Fetuses/Dam)	7±2.5	6±3.2	7±3.2	5±3.5	5±2.9
Total # Dead Fetuses	7	18	18	3	2
(Dead Fetuses/Dam)	1±1.1	1±1.9	1±1.3	0±0.6	0±0.8
Total # Resorptions					
Early [b]	12	3	9	16	12
Late [b]	0	0	0	0	1
Resorptions/Dam					
Early [b]	1±2.1	0±0.4	1±0.8	1±1.6	2±2.5
Late [b]	0±0	0±0	0±0	0±0	0±0.4
Litters with Total Resorptions	NA	NA	NA	NA	NA
Mean Fetal Weight (g)	34.14±5.232	35.86±6.565	32.95±8.154	32.44±5.205	31.57±0.964
Males	33.76±4.630	36.00±6.285	33.50±8.361	32.22±5.874	33.35±1.995
Females	34.41±5.933	35.32±7.833	32.28±8.151	32.80±5.495	30.81±1.154
Sex Ratio (% Male)	50	49	50	41	31

Observation	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Preimplantation Loss (%)	13	32	17	35	31
Postimplantation Loss (%)	N/A	N/A	N/A	N/A	N/A

a Data obtained from pages 28 and 29 in the study report.
b Calculated by ICF reviewers
NA - Not available; our reviewers cannot determine this information based on the how the individual animal data are presented.

B. DEVELOPMENTAL TOXICITY

1. External Examination: There were no treatment-related effects on gross external abnormalities. Observations include absent nares (one 5-mg/kg/day fetus), malpositioned eyes (one 5-mg/kg/day fetus), hypoplasia of the head (one 5-mg/kg/day fetus), misshapen head (one 5-mg/kg/day fetus and one 62.5-mg/kg/day fetus), herniated abdominal cavity (one 5-mg/kg/day fetus), and cleft palate (one 5-mg/kg/day fetus). External observations are summarized in Table 6a below.

2. Visceral Examination: There were no observations during the visceral examinations that are considered to be test-substance related. Dilation of the kidneys was the most common finding during the visceral evaluations, occurring in one 1-mg/kg/day fetus, two 5-mg/kg/day fetuses, two 20-mg/kg/day fetuses, and four 62.5-mg/kg/day fetuses. Although the fetal incidence of kidney dilation in the high-dose group was statistically significant compared to the control group, the observation is considered to be sporadic in nature. Other visceral observations include bifurcated renal artery (one control and one 1-mg/kg/day fetus), cor biloculare (one 1-mg/kg/day fetus and one 20-mg/kg/day fetus), herniated diaphragm (one 5-mg/kg/day fetus), and enlarged heart (one 5-mg/kg/day fetus). Visceral observations are summarized in Table 6b below.

3. Cephalic Examination: There were no treatment-related cephalic abnormalities. Sporadic cephalic observations include eye lens misshaped (one control fetus), vitreous chamber/optical cup misshapen (one control fetus), small diencephalons (one 5-mg/kg/day fetus), cerebral hemisphere missing (one 5-mg/kg/day fetus), and enlarged lateral ventricles (one 20-mg/kg/day fetus and one 62.5-mg/kg/day fetus). Cephalic observations are summarized in Table 6c below.

4. Skeletal Examination: There were no treatment-related skeletal abnormalities. A significant ($p \leq 0.05$) decrease in the percentage of fetuses with unossified sternebrae was observed at the 1- and 62.5-mg/kg/day dose levels (9.1% and 21.9%, respectively) when compared to the control group (25.5%), while a significant increase was observed at 20 mg/kg/day (44.3%). This increase is not considered treatment-related due to the lack of dose-response. Select skeletal observations are presented in Table 6d below.

TABLE 6a: External Examinations [a]

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
#Fetuses (litters) examined	103 (13)	77 (12)	105 (14)	61 (9)	32 (5)
#Fetuses (litters) affected	0 (0)	0 (0)	4 (4)	0 (0)	1 (1)
Absent nares	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Malpositioned eyes	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Hypoplasia of the head	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Misshapen head	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)
Herniated abdominal cavity	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Cleft palate	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)

a Data obtained from page 30 in the study report.

TABLE 6b: Visceral Examinations [a]

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
#Fetuses (litters) examined	103 (13)	77 (12)	105 (14)	61 (9)	32 (5)
#Fetuses (litters) affected	1 (1)	3 (3)	4 (3)	3 (2)	4 (2)
Bifurcated renal artery	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Cor biloculare	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)
Dilation of the kidney	0 (0)	1 (1)	2 (2)	2 (1)	4 (2)*
Herniated diaphragm	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Enlarged heart	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)

a Data obtained from page 30 in the study report.
* Statistically different ($p < 0.05$) from the control.

TABLE 6c: Cephalic Examinations [a]

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
#Fetuses (litters) examined	52 (13)	39 (12)	51 (14)	30 (9)	16 (5)
#Fetuses (litters) affected	1 (1)	0 (0)	1 (1)	1 (1)	0 (0)
Misshapen eye lens	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)

Observations	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
Misshapen vitreous chamber/optical cup	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Small diencephalon	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Missing cerebral hemisphere	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Enlarged lateral ventricles	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)

a Data obtained from page 30 in the study report.

TABLE 6d: Skeletal Examinations [a]

Observations [b]	Dose (mg/kg bw/day)				
	0	1	5	20	62.5
#Fetuses (litters) examined	102 (13)	77 (12)	105 (14)	61 (9)	32 (5)
#Fetuses (litters) affected	NA	NA	NA	NA	NA
Fontanel - Enlarged - Misshapen	 0 (0); 0% 0 (0); 0%	 0 (0); 0% 0 (0); 0%	 3 (3); 2.9% 1 (1); 1.0%	 0 (0); 0% 0 (0); 0%	 3 (2); 9.4% 2 (2); 6.3%
Thoracic centra -Dumbbell	 2 (2); 2.0%	 0 (0); 0%	 0 (0); 0%	 1 (1); 1.6%	 1 (1); 3.1%
Sternebrae - Unossified - Bipartate - Misaligned	 26 (11); 25.5% 4 (4); 3.9% 1 (1); 1.0%	 7 (6); 9.1% 5 (3); 6.5% 0 (0); 0%	 29 (10); 27.6% 1 (1.0);1.0% 1 (1.0);1.0%	 27 (8); 44.3%* 2 (1); 3.3% 3 (2); 4.9%	 7 (4); 21.9%* 0 (0); 0% 1 (1); 3.1%
Rib -Rudimentary-13 -Flying	 9 (7); 8.8% 6 (5); 5.9%	 6 (3); 7.8% 4 (3); 5.2%	 8 (6); 7.6% 3 (3); 2.9%	 3 (3); 4.9% 0 (0); 0%	 1 (1); 3.1% 1 (1); 3.1%

a Data obtained from pages 31-32 and 118-137 in the study report; results also provide percentage of fetuses affected.
*Statistically different from the control ($p<0.05$).
NA - Not available; our reviewers cannot determine this information based on the how the individual animal data are presented.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that oral administration of polyxylenol tetrasulfide (PXTS) and vehicle tricaprylin resulted in maternal toxicity (i.e., mortality and premature delivery) for all dams treated during gd 6 through 18, including the control dams. Treatment-related clinical observations include diarrhea, scant feces, no feces, cyanosis, and red urine. No other significant maternal effects were observed. Based on these results, the study author concluded that doses of PXTS ranging from 1 to 62.5 mg/kg/day in tricaprylin vehicle induced maternal toxicity in rabbits during organogenesis. There were no developmental effect observed; therefore, the NOAEL for fetuses is >62.5 mg/kg/day.

Additionally, the effects of tricaprylin observed in this rabbit study may be species-specific, since a previous study (1636/SN4) in which rats were dosed with up to 500 mg/kg/day of PXTS in tricaprylin reportedly did not produce any deaths over the 8-week treatment period.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Oral administration of PXTS in tricaprylin as well as tricaprylin alone (i.e., as the vehicle control) resulted in maternal toxicity (mortality, clinical signs, body weight loss, and premature delivery) in all study groups, including the control. The control group findings indicate that the vehicle, tricaprylin, may be a factor in the maternal toxicity observed in the treatment groups. In addition, the incidence of certain gross alterations appears to be treatment-related because they occurred primarily in animals that died prior to scheduled sacrifice. These changes include: mottled and pigmented lungs, red pigmented lungs, pale liver, red/brown pigmentation in the large intestine, fluid in the thoracic cavity, and various types of pigmentation in the lungs, liver, and large intestine. The pigmentation is consistent with a hemolytic effect of the test article, which was also observed in a 90-day oral toxicity study in rats (MRID 46062614).

Additionally, 62.5-mg/kg/day dams exhibited a decreased sex ratio and an increased preimplantation loss; these alterations did not reach statistical significance. High mortality in all groups, especially the 62.5-mg/kg/day group, resulted in a small sample size of litters/fetuses. It is likely that the change in the sex ratio is an artefact of the small sample size, given the lack of a treatment-related effect on most developmental endpoints (discussed below). For preimplantation loss, the lack of a clear dose-response relationship, as well as the small sample size, limited our reviewers' assessment; it is not possible to conclude if this effect was caused by administration of the test material. **The maternal LOAEL is 1 mg/kg/day; a maternal NOAEL cannot be determined because adverse effects (mortality, clinical signs, body weight loss, and premature delivery) occurred at all dose levels.**

2. **Developmental toxicity:**

a. Deaths/Resorptions: There were no treatment-related effects on fetal deaths or resorptions.

b. Altered Growth: Fetal growth was not altered by the test material.

c. Developmental Variations: There were no treatment-related developmental variations.

d. Malformations: There were no conclusive treatment-related malformations (external, visceral, cephalic, or skeletal); however, a statistically significant increase in the incidence of kidney dilation was observed at 62.5 mg/kg/day.

A significant increase in the incidence of kidney dilation was observed at 62.5 mg/kg/day. Without additional data on the metabolism of this compound and without histopathology data, however, it is not possible to conclude the mechanism of this effect or whether it is related to administration of the test article. The small sample sizes in this study also preclude a definitive

assessment of this effect. According to guidelines, at least 20 litters are needed to make proper comparisons. In this study there were only 13 control litters, twelve 1-mg/kg/day litters, fourteen 5-mg/kg/day litters, nine 20-mg/kg/litters, and five 62.5-mg/kg/day litters. **Consequently, the developmental LOAEL is considered to be 62.5 mg/kg/day for kidney dilation; the developmental NOAEL is 20 mg/kg/day.** There is considerable uncertainty regarding these values because of the small sample sizes. If historical data, provided by the study laboratory, indicated that the response at the high dose was within historical ranges for this effect, then the high dose could be considered a NOAEL.

C. STUDY DEFICIENCIES: The major study deficiency is the choice of vehicle. The vehicle appears to have been at least partially responsible for the maternal toxic effects. As a result, it is not possible to definitively relate the study findings to treatment with the test substance. In addition, the high level of maternal mortality and premature delivery in all study groups resulted in an insufficient number of dams and litters for analysis.

Other minor study design and/or reporting deficiencies are:

- The number of air changes in the study room was not provided.
- The study report does not state whether dosing was based on the most recently determined body weight.

D. STUDY CLASSIFICATION: This study is classified as **Unacceptable-guideline** and satisfies the guideline requirements for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits. Maternal toxicity was evident in all groups, including the control. The vehicle used (tricaprylin) likely was a contributing factor to the observed maternal effects. Nevertheless, the high level of maternal mortality and premature delivery in all study groups resulted in an insufficient number of dams and litters for analysis.