

12-21-93

December 21, 1993

Note to: Barry O'Keefe

From: Margaret Rice, TPTH Special Review Manager

RE: TPTH Rabbit Dermal Developmental Study submitted 8/31/93
under FIFRA section 6(a)(2)

HED has completed their review of the above-referenced study. Their review is attached. They have determined that the reported "equivocal findings" for certain external malformations noted at 3.0 mg/kg/day dose level are not considered sufficient to conclude a definite relationship to the test material.

The 6(a)(2) issue related to this data submission should be considered to be resolved. Please call if you have questions.

429091-01

Reviewed by: John Doherty, Ph.D.
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Marion Copley, DVM
Section IV, Toxicology Branch I (7509C)

Handwritten: 10/17/93
Handwritten: Marion Copley 10/29/93

DATA EVALUATION REPORT

STUDY TYPE: 83-3. Dermal Developmental Toxicity - rabbits

MRID NO.: 429091-01

TOX. CHEM. NO.: 896E

PC No.: 083601

TEST MATERIAL: Triphenyltin hydroxide (TPTH). From Lot
GFRAM911K. Technical grade active ingredient
stated as being 96% pure.

STUDY NUMBER(S): WIL-160012

SPONSOR: Elf Atochem North America

TESTING FACILITY: WIL Research Laboratories, Ashland, Ohio

TITLE OF REPORT: "A Developmental Toxicity Study on Triphenyltin
Hydroxide (TPTH) Administered Dermally in Rabbits"

AUTHOR(S): Mark D. Nemec

REPORT ISSUED: August 27, 1993

STUDY DATES: Approximately February 2, 1993 to August 16, 1993.

CONCLUSIONS:

NOEL (maternal and developmental toxicity) \geq 3.00 mg/kg/day.
Equivocal findings for certain external malformations noted at
3.0 mg/kg/day are not considered sufficient to conclude a
definite relationship to the test material.

Strain: New Zealand White Rabbit. Dose levels tested: 0, 1.5,
2.25 or 3.0 mg/kg/day in 1% carboxymethylcellulose.

Classification: CORE-GUIDELINES. No additional series 83-3
dermal developmental toxicity data are required for TPTH at this
time.

Quality Assurance Statement: Provided.

Good Laboratory Practice Statement: Provided.

REVIEW

Experimental Constants:

Test Chemical: Technical grade triphenyltin hydroxide (TPTH): Stated as being a fine white powder of 96% purity and from Lot GFRAM911K. The test material was suspended in 1% carboxymethylcellulose (CMC, Sigma Chemical Company). The 1% CMC was prepared by heating deionized water and the CMC added (10 gm/liter) and stirred until clear. TPTH was suspended in the CMC mixture by means of a Polytron PT 6000 mixer for approximately 5 min. The material was kept suspended by means of a magnetic stir bar. TPTH mixtures were said to be prepared daily.

Test System: New Zealand White Rabbits were obtained from the Hazleton Research Products Inc. Denver, Pennsylvania. Two shipments were obtained, one group on January 19 the other on February 16, 1993. They were stated as being 5 months old on receipt and varying in weight from 1943 to 4063 gms. The does were housed individually and artificially inseminated with sperm from stock bucks.

Basic Experimental Design: Four groups of 25 does that were artificially inseminated were dosed dermally as either control (CMC), 1.5, 2.25 or 3.0 mg/kg/day of TPTH in CMC. The test material was applied dermally on days 7 through 19 of gestation. The does were euthanized on day 29 of gestation and their uterine content assessed.

The dermal applications were made to a series of four quadrants on the shaved back of the rabbit. Each daily dose was applied on a rotating basis to the first site (designated as A and at the left lateral scapular area) and then on the next day to site B (right lateral scapular area) and then on the third day to site C (left lateral posterior area) and on the fourth day to site D (right lateral posterior area). On the fifth day the start of the cycle was repeated. This method was used to minimize the irritant effects of applying all of the TPTH to one area of the skin. Following application the test material was scheduled to be kept in place for 6 hours. The test material was kept in place by means of a 2" X 2" inch square gauze and elastic (ACE[®]) bandage which was further secured with Elastoplast[®] dermal tape. Elizabethan collars were used to prevent the rabbits from removing the bandages. Following the allotted six hours of application, the bandages were removed and the area washed with a soap and dried.

Analytical Chemistry: TPTH was assessed for in the dosing solutions by means of HPLC using UV detection at 210 nm. The following aspects were investigated.

Stability: Samples of 2, 4 and 20 mg/ml (dose levels prepared for the pilot study) were assessed after 24 hours of storage at room temperature. These yielded 102, 113 and 106% of their nominal concentrations after storage indicating that the test material was stable. Samples of 6, 9 and 12 mg/kg (samples prepared for the main study) yield 96.5, 99.3 and 109% of the nominal value when stored for 24 hours in the refrigerator.

Homogeneity: Samples of 6, 9 and 12 mg/ml of TPTH were assessed for homogeneity by sampling at the top, middle and bottom of their containers. The 6 mg/ml sample had the widest variation being only 4.7 mg/ml at the bottom and 5.88 mg/ml at the top. This sample had only 89.6% of the nominal concentration accounted for. The 9 mg/ml sample had variation between 7.91 mg/ml in the middle and 8.63 mg/ml at the top with 91% recovery. The high dose group of 12 mg/ml had a low of 12.2 mg/ml in the middle and 13.4 mg/ml at the

bottom with 106% recovery.

Concentration: Dose concentrations for the 6, 9 and 12 mg/ml samples for the three separate preparations were assessed for concentration. All samples were assessed to have > 100% of the nominal concentrations. Some had as high as 116% of the nominal concentration. Thus, sample are considered to have acceptable levels of test material.

Statistics: The following statistical tests were reportedly used to assess for differences in the data. All analysis were reportedly conducted using two-tailed tests for a minimum significance level of 5% when comparing the treated group to the vehicle control group.

Statistical Test	Parameters Investigated
Chi-square test with Yate's correction factor	Fetal sex ratios
Fisher's Exact test	Malformations and variations
Mann-Whitney U-test	Early and late resorptions Dead fetuses Postimplantation losses Mean litter proportions of malformations and variations
ANOVA (two-tailed) with Dunnett's test	Corpora lutea Total implantations Viable fetuses Fetal body weights Maternal body weights Weight changes Maternal net body weight changes Gravid uterine weights Maternal food consumption
Kruskal-Wallis test	Litter proportions of intrauterine data considering the litter rather than the fetus as the experimental unit.

Results

A. Maternal Toxicity.

1. Deaths and clinical signs. There were no deaths or clinical signs except for site of application dermal reactions.

Local site of application dermal reactions were present in all three groups dosed with the TPTH but not in the control group. Table 3 (attached, photocopied from the study report) illustrates the dermal reactions. The difference between the three groups dosed with TPTH indicated that the two highest dose groups were more affected than the lowest test dose group.

Overall, however, TB-I does not consider the differences in degree of effect to be consequential to the interpretation of the study.

2. Body Weight and Food Consumption. The study report asserts that there were no compound related effects on either body weight gain or food consumption. TB-I concurs with this evaluation. In particular, the mean body weight gain for days 7-19 when the animals were being dosed was 286 ± 95 , 311 ± 110 , 296 ± 111 , and 292 ± 194 for the control, low, mid and high dose groups respectively. Although the high dose group has a large standard deviation, the several intervals reported all had similar mean weight gains. The large standard deviations were noted in the high dose group but were also present in the control and other dose groups.

B. Uterine Data. Table 1 summarizes the efficiency of the study based on pregnancy and uterine data. No effects of treatment are indicated in this table.

Table. Uterine Data in rabbits dosed dermally with TPTH.

Parameter	Dose Level (mg/kg/day)			
	Control	1.5	2.25	3.0
Does on study	25	25	25	25
Gravid	19	22	20	21
With Viable fetuses	17	20	18	20
Aborted	1	0	0	1
With resorptions only	1	2	2	1
Viable fetuses	117	128	111	124
Mean # fetust s.d./dam	6.9 ± 2.67	6.4 ± 3.28	6.2 ± 2.94	6.2 ± 2.19
Post implantat- ion loss and mean \pm s.d	9 0.5 ± 0.79	16 0.7 ± 0.94	15 0.8 ± 1.48	17 0.8 ± 1.08
Fetal Weight (gms)	46.2 ± 6.53	49.2 ± 7.90	50.4 ± 4.92	47.8 ± 6.06
% males	47%	50%	50%	58%

Data from Tables 9 and 10, page 58 and 59 of study report.

1. Total of early and late resorptions.

TB-I notes that the high dose group has more males than females. The historical control data provided for 71 studies with this strain of rabbit indicated that the 58% figure obtained for male fetuses was well within the range for males.

C. Fetal Data.

1. Visceral and Soft Tissue Effects. The fetuses were reportedly examined internally and visceraally by a method that was described as a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major vessels. In addition, fetal kidneys were reportedly examined and graded for renal papillae by a method developed by Woo and Hoar.

A. Malformations: Table 2 illustrates the findings for malformations resulting from the external and visceral examination.

Table 2. External and visceral malformations in rabbit fetuses from does dosed with TPTH dermally.

Lesion	Dose Level (mg/kg/day)				
	Control	1.25	2.25	3.0	Historical Con. ¹
Fetuses/Litters	117/17	128/20	111/18	124/20	
.....
<u>External Effects:</u>					
Net Observed-total fetus/litter affected	1/1	1/1	0	8/6	
Adactyly	0	0	0	1/1	
Brachydactyly	0	0	0	2/2	Never recorded
Malpositioned forelimbs	0	0	0	3/2	Never recorded
Carpal/tarsal flexure	1/1	0	0	4/3 3.2%/15%	2.7%/7.1%
Hydrocephaly-external	0	0	0	4/2 3.2%/10%	3.0%/5.6%
.....
<u>Internal Effects -</u>					
Malformations					
Net Observed	1/1	1/1	2/2	2/2	
Hydrocephaly	1/1	1/1	1/1	2/2	
Variations:					
Accessory spleen	12/8 10%/47%	23/10 18%/50%	16/10 14%/56%	24/13 19%/65%	14.5%/55%

Data are from Table 11 of the study report (page 62).

None of the lesions reached a level of statistical significance. A statistical trend for increases can, however, be demonstrated.

1. The historical control data base provided by the testing laboratory (Appendix E, page 463) reported the results of 71 studies for the years 1982 to 1992. The percentage presented is the maximum of the range.

Among the high dose group fetuses, the following six fetuses from five does were identified as having multiple malformations:

- 15343-03 - adactyly and brachydactyly
- 15360-05 - carpal and/or tarsal flexure and hydrocephaly with or without domed head
- 15368-07 - malpositioned forelimbs and hydrocephaly (on visceral examination)
- 15369-03 - malpositioned forelimbs, carpal and/or tarsal flexure, hydrocephaly with or without dome head and narrow pelvic girdle with hindlimb rotation
- 15369-06 - malpositioned forelimbs, carpal and/or tarsal flexure, hydrocephaly with or without dome head
- 15385-04 - 9 malformations: brachydactyly, carpal and/or tarsal flexure, localized fetal edema, open eyelid(s), pinna smaller than normal (never recorded in historical control data base.) malpositioned digits, cephalocele, microphthalmia and/or anophthalmia, cleft palate.

Two other fetuses in the high dose group had singular incidents of umbilical herniation of the intestine and hydrocephaly with or without domed head. Neither of these malformations were noted in the control group. It was also noted that a low dose group fetus had a meningocele (fully described in the study report), a malformation that was also not previously reported in the historical controls.

B. Variations. There was also noted an apparent increase in pups with accessory spleens (refer to Table 2 above) as indicated in the 1.5 and 3.0 mg/kg/day dose groups. The high dose group exceeded the historical control range for both litters and fetuses affected, the low dose group exceeded the historical control range for fetuses only. The study report did not consider the apparent increase to be compound related "due to the similar incidence noted in the control group". In contrast, TB-I recognizes that the incidence in the high dose group is not similar to the control group (there are twice as many fetuses affected) but does not consider the variation to show a meaningful dose response over the range of 1.5, 2.25 and 3.0 mg/kg/day to justify that it is conclusively related to the material.

2. Skeletal Effects. Following fixation in alcohol, each fetus was macerated in KOH and stained with Alizarin Red S by the method of Dawson.

There were no obvious skeletal effects of TPTH treatment as indicated by there being 10, 5, 7 and 2 fetuses in the control to high dose groups affected with skeletal malformations. An "extra site of ossification anterior to sternebra No. 1 occurred in 6, 3, and 7 fetuses in the control to mid dose group respectively to account for many of the

malformations. The percentage of fetuses/litters with "skeletal variant sternebrae with threadlike attachment (4.0%/15%) in the high dose group was reported to be slightly higher than the maximum value observed in the historical control data bank (3.8% for fetuses). This variant was not noted in the control group but was present in 3.1%/15% and 2.7%/5.6% of the fetuses/litters for the low and mid dose groups. The study author notes that a lack of dose response suggests that this variant is not treatment related. TB-I concurs with this assessment.

The malformations noted on external examination of carpal and/or tarsal flexure, narrow pelvic girdle with hindlimb rotation and malpositioned forelimbs and digits were not always supported by skeletal findings in the same fetuses although purview of the original individual fetal effects indicated that they were confirmed viscerally.

Conclusion and Discussion (fetal effects). The testing laboratory asserts that the external and visceral findings for this study are equivocal and later states the NOEL and LELs for developmental toxicity are 2.25 and 3.0 mg/kg/day. The external effects as indicated above are present in the high dose group and in some cases in excess of the historical control limits noted in 71 different studies and in other cases were not even noted in the historical control data base. TB-I considers that these apparent increases in external malformations although verified by visceral examination but not always by skeletal examination are insufficient to declare that the 3 mg/kg/day dose level is a true developmental toxicity LEL. In support of this position, TB-I notes the following:

i. There was no effect on fetal body weight. Decreased fetal body weight was determined to be the most sensitive indicator of developmental toxicity in three species (rat, rabbit and hamster, refer to the TPTH Developmental Toxicity Peer Review packages for the Peer Review meetings dated April 5, 1990 and June 13, 1990)) following oral administration. The lack of an effect on body weight suggests that TPTH never actually reached the fetuses in utero.

ii. Malformations of the same description were not also noted in the oral rabbit developmental toxicity study (refer to HED Document No.: 005917, MRID No.: 401048-01) which showed evidence of maternal toxicity (decreased gestational weight gain) and developmental toxicity (decreased pup weight and possible decreased ossification of the hyoid). The presence of maternal toxicity and developmental toxicity in the oral study indicated absorption of the test material. The dermal study did not indicate any decreased ossification of the hyoid.

iii. The external malformations do not reach statistical significance.

iv. The dose levels are very close together and TB-I considers that a

test chemical effect noted at the high dose group of 3.0 mg/kg/day should also have at least some effect at the next lower dose of 2.25 mg/kg/day. Based on the similarity of the dermal reactions to treatment, TB-I considers that there is no meaningful pharmacological difference between the dose levels 2.25 and 3.0 when applied dermally.

v. The variety of the external malformations noted in the high dose group do not suggest a common target organ or pattern of developmental toxicity. On the contrary here, it is noted that of the six fetuses in the high dose group that had the external malformations involving the skeletal system (confirmed or otherwise) all were males indicating that the male fetus is a common target. This fact is noted but does not convince TB-I that the effect is test chemical related.

vi. The total of pups/litters noted to have skeletal, soft tissue and external malformations was about equal in all test groups being 10/4, 6/4, 9/5, and 11/8 in the control, low, mid and high dose groups respectively. For example, although the high dose group had more incidents of pups with external malformations (8) than the control (1), the high dose group (2) had less skeletal malformations than the control group (10). On a litter basis, although there were more litters affected in the high dose group (8) than in the control (4), some of these litters had only a single pup with a malformation.

Although these justifications when considered individually may not be sufficient to dismiss the findings, TB-I considers that these justifications taken all together are the basis for concluding that the malformations are unlikely related to the test article administration. These incidents are noted and discussed as above but should not be included in the conclusions for definite effects of the test material.

CONCLUSION (Study): This study is CORE GUIDELINES. The following "one liner" is supported.

LEL (maternal and developmental toxicity) > 3.00 mg/kg/day. Equivocal findings for certain external malformations noted at 3.0 mg/kg/day are not considered sufficient to conclude a definite relationship to the test material.

Strain: New Zealand White Rabbit. Dose levels tested: 0, 1.5, 2.25 or 3.0 mg/kg/day in 1% carboxymethylcellulose.