REVIEWER

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 8340-15-Triphenyltin Hydroxide (TPTH): Review of 21-Day Dermal Toxicity study (rats); Teratology Study with Development Phase (rats); Dermal Sensitization Study (guinea pigs)

TOX CHEM NO. 896E

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Background:

The American Hoechst Corporation (Somerville, New Jersey) has submitted four studies to support continued registration of triphenyltin hydroxide (TPTH). The reviews of three of these studies are presented in this memo. Review of the fourth study (dermal penetration) is incomplete at this time.

Since no specific registration request was made with this submission, the studies were reviewed and no specific use of TPTH is addressed in this memo.

Comments:

1. The dermal sensitization study was not acceptable. Additional studies will have to be submitted. Because the study submitted indicated positive effects other study types designed to assess sensitization should also be conducted. The registrant is referred to EPA's guidelines for seven study types suggested.

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2. The teratology study indicated small kidney weight differences (4.5-7.5%) without associated pathology and no obvious functional changes. Although the weight differences were disturbing, the lack of supporting pathological findings and functional (as assessed only by urine volume) changes and because the weight difference was small, TB is not concerned with a teratogenic effect of TPTH. At worst, the weight difference is a fetotoxic effect.

3. The 21-day dermal toxicity study was found to be ACCEPTABLE.

4. The dermal absorption study is currently under review and the expected completion date is mid September, 1985.

Studies Reviewed

Study	Result	Classification
21-Day Dermal - rats	NOEL > 20 mg/kg/day (HDT) for systemic effects < 5 mg/kg/day for local irritation.	Guideline
Dermal Sensitization - guinea pigs	Study indicates that TPTH is a "very slight sensitizing agent."	Not Acceptable
Teratology (with development phase) - rats	Maternal toxicity: NOEL = 1.00 mg/kg/day LEL = 2.80 mg/kg/day-body weight changes; at 8.0 mg/kg/day, hair loss and lethargy. Teratogenic: NOEL > 8.0 mg/kg/day (HDT) Fetotoxic: NOEL = 1.0 mg/kg/day LEL = 2.8 mg/kg/day, kidney weight changes. ~~LEL~~ At 8.0 mg/kg day-poor pup survival at birth.	SUPPLEMENTARY

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Sensitization Study-Guinea Pigs

A. Skin Sensitization Study in Albino Guinea Pigs with Triphenyltin Hydroxide (Code HOE29664 OF ZD97 001 Technical Substance). Wil Research Laboratories, study number Wil-39019, date of issuance June 3, 1985, EPA Accession No. 258230, Tab C2.

B. The test material was triphenyltin hydroxide (TPTH). It was stated as being 97.1 percent pure and had the code number HOE 029664 OF ZD97 001. The test material was made to a concentration of 1.0 percent w/w mixture in distilled water. Note: Since TPTH is insoluble in water, the test solution was a suspension of the material in water.

C. The test animals used were Hartley strain albino guinea pigs obtained from Kuiper Rabbit Ranch, Gary, Indiana. The study consisted of a preliminary irritation study designed to test for primary irritation in order to assist in selecting dose levels for the sensitization study. In the preliminary study, the guinea pigs were dosed with 0.1, 0.25, 0.5, and 1.0 percent TPTH in distilled water and monitored for primary reactions. The high dose level of 1.0 percent which produced some signs of irritation was selected for the induction phase, the lower dose level of 0.1 percent was selected for the challenge phase.

The induction phase of the study consisted of 10 males and 10 females with either TPTH (1.0% in water); 5 males and 5 female dosed with the positive control 0.25 percent dinitrochlorophenol in 80 percent ethanol. Three induction doses were applied which were spaced 1 week apart. The induction doses were applied at the left shoulder area of the guinea pigs. Note: There were no indications as to how the guinea pigs were prepared for dosing prior to application of the induction material.

The challenge phase consisted of a single application of the prepared test or positive control agent to previously unexposed areas of the skin 13 days after the last (third) induction dose. The rechallenge phase was made 7 days after the first challenge.

The test doses were applied by placing the dose (0.4 ml) on a pad of ParkeDavis-Readi-Bandage which was then applied to the guinea pig and then occluded with dental dam and secured with nonirritating tape. The test materials were kept in place for 6 hours.

The guinea pigs were dipilated with Neet® Dipilatory Lotion 3 hours prior to evaluating the skin reactions.

This test method is a modification of the method of Buehler.

D. Results

1. Primary Skin Reactions

Grade 1 only reactions were noted (slight confluent or moderate patchy erythema). The highest or most serious response was in the group receiving 1.0 percent TPTH.

2. Induction Phase

Grade 1 reactions were reported in some guinea pigs dosed with TPTH. Only very slight reactions were reported for the guinea pigs receiving the positive control.

3. Challenge and Rechallenge Phases

The following two tables, as they appeared in the study report, indicate the results of this study in terms of irritation indices.

Irritation Indices

The irritation indices are calculated based on the skin reactions obtained following the challenge and rechallenge dosings.

a. Incidence Index

The incidence index is the number of animals having responses of grade 1 or greater at either 24 or 48 hours divided by the number of animals in the group.

	Initial Challenge	Rechallenge
Test Group	0.20	0.15
Naive Control Group	0.00	0.00
Positive Control Group	0.20	0.30

b. Severity Index

The severity index is the sum of the test grades divided by the total number of animals in the group. This index is calculated for each group separately at 24 and 48 hours. Grades of ± are equal to 0.5 for calculation purposes.

	Initial Challenge 24H	Initial Challenge 48H	Rechallenge 24H	Rechallenge 48H
Test Group	0.60	0.50	0.53	0.35
Naive Control Group	0.35	0.20	0.40	0.40
Positive Control Group	0.55	0.55	0.65	0.45

On this basis the test material, TPTH, is considered to have caused sensitization of the skin as evident by both both incidence and severity indices which are equivalent to the positive control group.

E. Conclusion

The study report concluded that the test material (TPTH) is a "very slight sensitizing agent" on the albino guinea pig under the conditions of this study.

This study is NOT ACCEPTABLE. The borderline response obtained is not sufficient by itself to classify this chemical as a skin sensitizer. Additional studies using one or more of the other methods to assess skin sensitization must be conducted. In the future studies, the positive control must give a positive response.

Dermal Dosing study-Rats.

A. 21-Day Dermal Toxicity Study in Rats with Triphenyltin Hydroxide (Code HOE29664 OF ZD97 001 Technical Substance). Wil Research Laboratories, study number WIL-39018, date of issuance June 4, 1985, EPA Accession No. 258230, Tab C3.

B. The test material was triphenyltin hydroxide. It was stated as being 97.1 percent pure and was identified as HOE029664 OF ZD97 001. No adjustment was made for the impurities when the test material was prepared for application. The test material was described as a white powder. The vehicle used for this study was distilled water. Sampling during the experiment indicated that the suspensions ranged from 87.2 to 101.0 percent of the desired dosing levels.

C. The test animals were Crl:CD® (SD)BR rats which were obtained from the Charles River Breeding Laboratories, Portage, Michigan. The rats were grouped into 4 groups to receive the test material at 0 (control), 5, 10, and 20 mg/kg/day; each group consisted of 10 males and 10 females. The rats were prepared for the dermal application by shaving 10 percent of

their body surface on their backs. The test material (as a suspension in distilled water) was applied to their backs at a "shaved, unabraded" test site on a daily basis for a total of 15 applications (5 times per week for 3 weeks). The test material was kept in place by a gauze dressing covered with impervious plastic wrap and kept in place for 6 hours. After 6 hours of exposure, the dressing and excess test material were removed and the site washed. The control rats were treated in a similar manner except that distilled water only was used.

D. Survival. None of the rats in the study died.

E. Reactions. Local (site of application) reactions were the only treatment-related reactions noted. All dose levels of rats treated with TPTH showed signs of erythema, edema, scabbing, atonia, fissuring and/or blanching. Some of these signs were evident on the second day of dosing and onwards (see also histopathology comments below).

NOEL < 5 mg/kg/day (local reactions only).

F. Body Weight. Only slight changes in body weight were evident and similarly no meaningful changes in food consumption were noted.

NOEL > 20 mg/kg/day (HDT).

Note: For sections G, H, and I below, samples were taken at pretest and just prior to sacrifice. Blood was withdrawn from 10 rats of each sex from the orbital sinus for the pretest and from the abdominal aorta for the terminal sacrifice. Only the data for the terminal sacrifice groups were presented in the study report.

G. Hematology. Evidence was presented that determinations were made on white blood cells (total and differential), red cells, hemoglobin, hematocrit, mean cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. The test report asserts that no compound-related effects were noted.

NOEL > 20 mg/kg/day (TB concurs with the test report).

[Note: In other rat studies, the white blood cells were depressed following oral administration. See the Registration Standard for reviews and citations].

H. Clinical Chemistry. Evidence was presented that determinations were made on glucose, urea nitrogen, creatinine, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, phosphorous, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin, cholesterol, and globulin. The study report asserts a NOEL of > 20 mg/kg/day.

Although some deviations from the control data were evident when the high dose groups (male and female) were compared with the control unit (i.e., glucose levels, SGPT, A/G ratio), the magnitude, together with the large standard deviations of the data, do not necessitate a compound-related effect.

NOEL > 20 mg/kg/day (HDT).

I. Urinalysis. Evidence was presented that determinations were made on urine volume, color, appearance, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrogen, urobilinogen, and microscopic evaluations. The study report states that there were no compound related effects.

NOEL > 20 mg/kg/day (TB concurs with the study report).

It is noted that urine volume for the high dose group males was only 50 percent of the control group. In the absence of supporting information such as body weight differences and pathological changes, this difference is not considered to be a response to the test material.

J. Organ Weight. Evidence was presented that the brain, testes/ovaries, seminal vesicles, prostate, adrenal glands, kidney, spleen, liver, heart, thymus gland, lung, thyroid gland, and pituitary were weighed. Absolute, relative to body weight, and relative to brain weights were determined. The test laboratory asserts that no compound-related changes in organ weights were evident.

NOEL > 20 mg/kg/day (TB concurs with the study).

Note: The thyroid and thymus glands showed decreased weight in the lowest dose group, but these differences are not considered compound-related.

K. Gross Pathology. There were only two internal organs for which gross necropsy revealed lesions. These organs were the kidney which was described as "pitted." There were two incidences in the male groups - one each in the control and high dose group. Among the females there were none in the control, but 1, 2, and 1 in the low, mid and high dose groups. The other organ was the testes - one mid dose group male had this organ described as small.

The treated areas of the skin had areas described as "thickened-exfoliated, ulcerated" and "exfoliated in all groups treated with TPTH." One female in the mid dose group had "blanching" of the treated area.

NOEL > 20 mg/kg/day for systemic effects (see histopathology for kidney discussion).

NOEL < 5 mg/kg/day for local effects at the site of application.

L. Histopathology. The protocol called for microscopic examination of the tissues from the control and high dose test groups. Only the skin, bone marrow, and gross lesions from the low and mid dose groups were scheduled for examination. Dr. Robert Geil, D.V.M., A.C.V.P., was the pathologist responsible for the evaluation. Evidence was presented that the adrenal, bone/bone marrow, brain, epididymis, heart, kidney, liver, lung, lymph node, parathyroid, pituitary, prostate, seminal vesicle, skin, spinal cord (cervical and thoracic), spleen, testis, thymus, thyroid, ovary, and oviduct were examined. The test report asserts that only the local site of application indicated material related effects. No systemic effects were evident. Some comments on the individual organs are as follows.

(i) Liver. The high dose group females (but not males) had four incidences of "hepatocyte vacuolation, multifocal minimal"; the control group had the lesion described as "generalized" rather than "multifocal."

(ii) Kidney. There was a large difference noted in urine volume among the males and the kidneys were described as "pitted" in the females at gross necropsy. No compound-related changes were noted by microscopic examination.

(iii) Thymus. The low dose group was lighter in weight. No compound-related changes were noted by microscopic examination in either sex. Signs of hemorrhage were found in both controls and high dose group. The low dose group was not examined.

(iv) Thyroid. The low dose group was lighter in weight. The low dose group was not examined microscopically. The high dose male group (but not the female) had 4 incidences of 10 rats of "ultimobronchial cyst," but there were more in the control group.

(v) Local Skin Reactions. The major local reactions were described as "acanthosis, chronic dermatitis, hyperkeratosis, and parakeratosis. Some of these were in the control also (probably as a result of the water and dressing), but the intensity was more pronounced in the rats treated with TPTH. The pathologist stated that these lesion types would disappear following removal of the test material.

NOEL > 20 mg/kg/day - for systemic effects.

NOEL < 5 mg/kg/day - for local irritation effects.

M. N/A

N. This study is CORE GUIDELINES. The NOEL's are as follows:

NOEL > 20 mg/kg/day (HDT) - for systemic effects.
NOEL < 5 mg/kg/day - for local irritation (acanthosis, chronic dermatitis, hyperkeratosis) considered reversible lesions.

Teratology With Development Phase

A. Study Type - Teratology (Study title: One-Generation Teratology and Reproductive Study in Rats with Triphenyltin Hydroxide (Code HOE029664 OF ZD97 001 Technical Substance), Wil Research Laboratories, Study number WIL-39013, date of Issuance June 4, 1985, EPA Accession No. 258229.

Note: Although the study title includes "Reproductive Study" neither the study design nor the data presented are appropriate for a reproduction study and this study is thus reviewed only as a teratology study.

B. The test material was triphenyltin hydroxide (TPTH) and was stated as being 97.1 percent pure. The code number was HOE029664 OF ZD97001. No adjustment was made for [redacted] The test material was prepared in Mazola® corn oil prior to animal administration. The stock test material preparations were analyzed to assure that the desired concentrations were obtained.

C. The test animals used were Crl:CD®(SD)BR rats. Five groups of 25 mated females were dosed as either 0.00, 0.35, 1.00, 2.80 or 8.00 mg/kg/day of TPTH. Dosing was by gavage on days 6 to 15 of gestation. The pregnant dams were allowed to deliver their pups naturally. Those dams which had not delivered by day 25 were sacrificed and examined for signs of pregnancy status.

D. Maternal Effects: None of the rats died. The study report asserts a NOEL for effects on the dams of 1.00 mg/kg/day. The only reported effect at 2.80 mg/kg/day was body weight gain which was "probably inhibited to a slight degree." Inspection of the data indicates that the dams in the group receiving 2.8 mg/kg/day were slightly higher than the concurrent control group. However, when compared with dams from a control group run previously with the same strain of rat and from a study in which TPTH was tested revealed a slight weight loss during dosing. In the previous study, the dose level of 2.80 mg/kg was determined to cause weight loss. Thus, the NOEL of 1.0 mg/kg/day is set to be consistent with other studies with this chemical.

Other signs reported at 8.0 mg/kg/day included "hair loss," lethargy, and yellow staining of the urogenital area during the treatment period. Gestation length was also increased significantly ($p < 0.1$ and by an average of 0.8 day or 3.7% longer). This may have been due to a single female which finally delivered on day 24. This female delivered pups still in their amniotic sac or were cannibalized by the dam.

E. Litter Data. There were 261, 326, 327, 312, and 163 live pups for the control, low, mid-1, mid-2, and high dose groups, respectively. The high dose had the most dead pups (36 or 18% of total vs only 25 or 8.7% of the total in the control group.

There were 22, 23, 24, 23, and 14 live litters for the control to high dose groups respectively. The low number of pups and litters in the high dose group is related to the fact that there were seven nongravid females in this group vs three in the control group. Also, both the control group and the high dose group had five litters that were "total litter loss" after delivery.

The sex ratio was 0.76 for the control group and 1.33 for the high dose group meaning that there were more males in the high dose group. However, the report considered this difference to be unmeaningful because of the low male to female ratio in the control group.

The mean litter size (weight) for the control group was less than that expected for this strain of rat when compared to available control data. This led to complications in interpreting the weight changes in the dosed groups. When compared to expected weight for controls (as derived from other studies), the high dose test group could be shown to have lower weight.

Since all of the pups selected for development through lactation survived the 28 days, no effect of TPTH on survival through lactation was evident.

> Note: The study design did not call for typical preparation of visceral and skeletal examination as would be expected for a teratology study.

F. Hematology, Serum Chemistry. On lactation day 28, 4 pups per litter (2 males and 2 females) were selected and comprehensive hematological (9 parameters plus differential WBC counts) and serum chemistry (16 parameters including enzyme levels) determinations were made. The following changes were noted:

(i) Mean Corpuscular Hemoglobin - decreased (-4% and -3%) in both sexes at 8.0 mg/kg/day, but the values were still within the normal range for this strain of rat.

(ii) Lactic Dehydrogenase (LDH)- was decreased in all groups of males (-30%, -47%, -52%, and -62%) and females (-19%, -45%, -49% and -53%). The data for 1.00, 2.80, and 8.00 mg/kg/day group were statistically significant. Toxic responses to chemicals are often indicated by increases in lactic dehydrogenase, not decreases. Even though the control group (as well as some of the test groups) had high standard errors, the pattern of decreased enzyme activity suggests that TPTH is inhibiting the enzyme. No pathological state was found to correlate with the decreased enzyme activity.

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(iii) Calcium - Ca^{++} levels were increased for males receiving 1.0 (+16.8%), 2.80 (12.3%) and 8.0 (18.3%) mg/kg/day but females were not affected. Changes in Ca^{++} levels would theoretically be an anticipated effect of TPTH because this agent prevents binding of Ca^{++} to membranes.

(iv) Serum Glutamic Oxaloacetic Transaminase (SGOT) - was also decreased in a dose-related manner for females for all dose levels (-14%, -21%, -24%, -35%). Males were not affected.

The significance of decreased SGOT is not known and no pathological state was indicated to cause this decrease. Cell injury is often associated with increases in SGOT.

(v) Globulin - levels were decreased in females (-19%, -17%, -24%, and -32%), only the values at 2.80 and 8.00 mg/kg/day were statistically significant.

Note: The changes in LDH, Ca++, SGOT and globulin are considered by this reviewer to be incidental and not directly related to TPTH. For example, the last application of TPTH was given to the mothers of the rats tested some 38-40 days prior to when the assay for blood chemistries was made. Such changes as noted in these parameters would not likely result from such an early application especially in the absence of pathology in any of the internal organs.

G. Urinalysis. The protocol called for assessment of 12 parameters, however, data on urine volume only were presented.

> Note: This ommission is an important deficiency because the kidneys were regarded as a possible target organ for a teratogenic/fetotoxic effect of TPTH.

Urine volumes for males ranged from 22 to 46 ml and the lowest volume was in the high dose test group but the highest volume was in the group receiving 1.0 mg/kg/day. In the females, the urine volumes ranged from 24 to 45 ml and again the high dose test group had the lowest volume but the two groups recieving 1.0 and 2.8 mg/kg/day had the highest volumes. Thus, although the high dose group had the lowest volume in both sexes, no consistent dose related effect of TPTH is evident.

H. Necropsy. No consistent pattern of compound-related effects were noted among dams failing to deliver, dams with total loss, dams examined after lactation day 21 (although the number of im-

plantation scars counted at necropsy and the number of pups born was increased for the high dose groups when compared to the control group), culled pups, and pups sacrificed at lactation day 21.

I. Organ Weights. The relative kidney weights for both males and females were statistically significantly elevated at the 2.80 and 8.0 mg/kg/day (4.5 to 7.3%). For males, the absolute weight was also elevated for the group receiving 1.0 mg/kg/day (+5.7%). [Note: Only the kidney weights were determined.]

J. Histopathology. The protocol called for examination of the kidneys of the pups sacrificed at 28 days. Dr. Ray Brown, (D.V.M.) was the pathologist responsible for assessing the slides. No lesions indicative of an effect of TPTH were reported.

Conclusion:

This study is CORE SUPPLEMENTARY. The SUPPLEMENTARY classification is related to the design of the study which did not include the conventional assessment of skeletal and visceral structures required for a teratology study. The study was especially designed to assess kidney effects.

Interpretation of the data in this study is confounded by the weight difference in the control group which was lower than the norm for this strain of rat. The data related to weight differences (although only 4.5 to 7.5%) in the kidney may or may not be a result of TPTH toxicity.

The NOEL assigned for this study are as follows:

Maternal toxicity

NOEL = 1.0 mg/kg/day.
LEL = 2.8 mg/kg/day - weight changes and at
8.0 mg/kg/day - hair loss and lethargy.

Teratogenic

NOEL > 8.0 mg/kg/day.

Fetotoxicity (in 28-day-old pups).

NOEL = 1.0 mg/kg/day
LEL = 2.8 mg/kg/day, kidney weight changes. ~~LEL~~ At 8.0 mg/kg/day- poor pup survival at birth.

[Note: Alkaline phosphatase and SGOT decreases and serum Ca++ and globulin levels decreases were also noted but these data were not interpreted as a tocicity response to TPTH. The rat chronic feeding study should be carefully assessed for similar changes.]