CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 896 E
WASHINGTON, D.C. 20460

SEP 27 1989 007501

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No.: 8340-17. Triphenyltin Hydroxide: Review of rat chronic feeding/ oncogenicity and mouse oncogenicity studies conducted by the RCC Laboratory and submitted May 1989. Indications of oncogenic effects in the pituitary of female rats, testis of male rats and liver in male and female mice.

TOX CHEM No.: 896E
TOX PROJECT No.: 9-1444
Record No.: 245204
MRID No.: 410857-01 (Mouse 5 volumes)
410857-02 (Rat 8 volumes)

FROM: John Doherty 9.25.89
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

TO: Susan Lewis
Product Manager #21
Registration Division (H7505C)

THROUGH: Karl Baetcke 9/25/89
Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Background

The Hoechst-Celanese Company has submitted a rat chronic feeding/oncogenicity study and a mouse oncogenicity study with the fungicide triphenyltin hydroxide (TPTH). These studies were reviewed by Toxicology Branch I (TB-I) and the DERs are attached. The following comments apply.

Toxicology Branch Comments

1. Both the rat (pituitary adenomas in females and Leydig cell tumors) and mouse (liver adenomas in both sexes and carcinomas in



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females) were determined to be associated with dose related increased incidence of neoplasms.

The issue of the oncogenicity of TPTH will be presented to the HED Peer Review Committee to determine the carcinogenicity classification and the need for quantitative oncogenic risk assessment. It is expected that this peer review meeting will be held in the fall (October or November) of 1989.

Recommendations regarding the regulatory aspects of TPTH with regard to carcinogenicity will be made pending completion of the Peer Review meeting.

1A. The study report discusses the oncogenic findings in terms of the increases tumors as being related to a promoter effect of TPTH. TB-I does not currently recognize that there are appropriate guidelines for distinguishing tumor promoters from direct acting oncogens. Because of the low dose levels involved in this study, TB-I current policies would not likely differentiate its regulatory recommendations whether or not TPTH were eventually proven to be a direct acting oncogen or a tumor promoter.

2. Both the rat chronic feeding/oncogenicity study and the mouse oncogenicity study have been classified as CORE GUIDELINE.

3. The rat chronic feeding study did not demonstrate a NOEL. At the lowest dose level tested (5 ppm or 0.4 mg/kg/day in females) there were increased deaths in the females apparently related to pituitary tumors.

Since the study did not demonstrate a NOEL and the toxic effect at the lowest dose level tested is serious (deaths), this study can be used for ADI setting only with a modifying factor in addition to the usual 100 fold safety factor.

It should be noted that it is possible that TB-I may require a second chronic feeding study designed to establish a NOEL in the rat. Since the deaths apparently result from pituitary tumors, the decision to request a second study is related to the outcome of the carcinogenicity Peer Review as well as the possibility of using some other study for ADI setting and/or using a modifying factor.

4. Special discussion of immunotoxicity.

The conclusion of the 90 day subchronic feeding range finding studies for both rats and mice was that TPTH caused decreases in immunoglobulins in both species at all dose levels tested. When these studies were reviewed it was stated that the need to base the NOEL on immunoglobulin decreases would be reevaluated when the chronic feeding and oncogenicity studies are

completed.

The immunoglobulin levels for some immmunoglobulins were decreased in both the rat chronic feeding and mouse oncogenicity studies at all dose levels. Other immunoglobulin levels were increased in a dose dependent manner (refer to DERs attached). According to Dr. Lynnard Slaughter, consulting pathologist, the blood levels of immunoglobulins depend upon many factors and have circadian rhythms. The immunoglobulin data for these studies is considered compromised because the serum total protein and albumin/globulin ratio should have been determined at the same time the immunoglobulins were quantitated. There were also no pathological correlates supporting the immunoglobulin decreases.

TB-I previously requested that special immunotoxicity testing with TPTH be provided by the registrant (refer to J. Doherty memo concerning the protocol review dated November 3, 1988). TB-I will reevaluate the issue of immunotoxic potential of TPTH pending receipt and review of the special immunotoxicity studies.

In conclusion, the decreases in immunoglobulins in the rat and mouse subchronic feeding studies, the rat chronic feeding study and mouse oncogenicity study are noted but they are not regarded by TB-I as <u>definite</u> indications of immunotoxic potential of TPTH.

5. Skeletal muscle atrophy and degenerative neuropathy in the sciatic nerve in rats.

Both the mid and high dose test group male rats had increased incidence of <u>skeletal muscle atrophy</u> (DER p. 15) and <u>degenerative neuropathy in the sciatic nerve</u> (DER p. 16). The study report does not regard these lesions as being a consequence of TPTH dosing. TB-I, however, considers that these lesions may be related to the general cell toxicity of TPTH. In order to resolve this issue, <u>the registrant is requested to provide historical control data for these lesion types</u>. This information should be presented in a manner similar to the tumor historical control data already provided in the study report.

In the meanwhile, TB-I will regard these lesions (skeletal muscle atrophy and degenerative neuropathy in the sciatic nerve) as being related to TPTH toxicity until the registrant submits the above historical control data and any other information which will provide a basis for TB to reconsider its position. [Note: The NOEL for this study is already set below the dose levels involving these lesions based on <u>deaths</u> in females.]

6. Analysis of tin in rat tissues.

This information has not been provided by the registrant at this time and the report states that these data will be submitted separately. Submission of these data are expected of the registrant.

007501

Reviewed By: John Doherty 9/20/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl Baetcke 9/21/89
Chief, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-1 and 2 - Oncogenicity and Chronic Feeding - Rats

MRID No.: 410857-02 (8 volumes) TOX Chem. No.: 896E

Test Material: Technical grade triphenyltin hydroxide, Batch Nos. HOE 029664 OF ZD097 0004 (97.2%, weeks 1 to 4) and 0007 (97.0%, weeks 5 to termination, from Lot No. NWRAM-805 K)

Synonyms: TPTH, Fentin hydroxid

Test Animals: Wistar rats, KFM-Han., outbred, Specific Pathogen Free-Quality. Obtained from KFM Kleintierfarm Madorin AG, Switzerland. They were about 5 weeks old at the start of dosing. They were housed in groups of five.

Study No.: 046980

Sponsor: Hoechst Celanese Corporation
Somerville, NJ

Testing Facility: Research Consulting Company (RCC), Itingen, Switzerland. Specialized assessments were made at other laboratories (refer to DER text)

Title of Report: TPTH Technical (Code: HOE 029664 of ZD97 0004) Chronic Toxicity/Oncogenicity 104-Week Feeding Study in Rats.

Author(s): H. Tennekes, K. Horst, H. Luetkemeier, W. Vogel, O. Vogel, B. Schlotke, H.A. Bhlers, E. Muller, Ch. Terrier

Report Issued: April 18, 1989

Conclusions:

Neoplastic. The pituitary (females) and testis have been identified as oncogenic target organs for TPTH in the rat. This information will be Peer Reviewed by HED for carcinogenicity classification of TPTH.

Systemic effects.

- NOEL < 5 ppm (<0.4 mg/kg in females). At this level there were increases in deaths and behavioral reactions in females probably associated with pituitary tumors. Decreases in immunoglobulins (IgG1, Ig2a, Ig2c, IgA).

- LEL = 20 ppm, decreases in body weight gain; decreases in liver weight; "cystoid change" in pituitary (males); nodules (pituitary, females) and compression of brain; liver bile duct proliferation and portal sclerosis. Skeletal muscle atrophy in males and degenerative neuropathy in the sciatic nerve (males, tentative conclusion, additional historical control data requested).

- LEL = 80 ppm, decreases in food consumption, increases in serum enzyme activity (ASAT, ALP, and ALAT), pituitary pars intermedia hyperplasia (males), Leydig Cell hyperplasia and testicular tubular atrophy, liver eosinophilic focus (females).

Classification: CORE-GUIDELINE

Special Review Criteria (40 CFR 154.7): Demonstration of the oncogenic effects in the pituitary (females) and testis (males) may require Special Review for TPTH.

Quality Assurance Statement:

A statement was provided that was signed for the Quality Assurance Manager (K. Schneider) by an individual whose signature was illegible. The statement indicated that at least 13 inspection reports were made. These reports, however, did not include the assessments of the immunoglobulins which were made at the ANAWA laboratories.

## Review

The basic design of this study consisted of four groups of 70 male and 70 female Wistar rats. Of these 70 of each sex, 50 were included in the main phase for oncogenicity evaluation and were dosed for 104 weeks; 10 were used for clinical evaluations at 26, 52, 78, and 104 weeks. The remaining 10 were an interim sacrifice (52 weeks) group.

The dose levels selected (0, 5, 20 and 80 ppm) were based on a 90-day subchronic feeding study (refer to review by J. Doherty dated July 15, 1986 for EPA Reg No. 8340-17). Analysis of food consumption and body weight data over the course of the 2-year dosing period indicated that the rats received the following intake of TPTH.

| | TPTH (mg/kg/day) | |
|---|---|---|
| | Males | Females |
| Controls | 0 | 0 |
| 5 ppm | 0.3 | 0.4 |
| 20 ppm | 1.3 | 1.6 |
| 80 ppm | 5.2 | 6.2 |

TPTH was added to the diet by mixing the chemical with microgranulated feed followed by pelleting. The pellets were deep frozen (-20 C) until ready for use. Fresh feed was offered to the rats daily. The mixtures were prepared twice monthly. The stability and homogeneity of TPTH in the feed was assessed by RCC UMWELTCHEMIE AG, Itingen, Switzerland, and the results were presented in an appended report (pages 708-725). This report concluded that TPTH is stable in the feed for 21 days. The mean concentrations of TPTH in the feed were in the range of 79.6 to 115.7 percent. The homogeneity was demonstrated to be -14 to +17 percent of the nominal concentration. Not all feed preparations were analyzed. For example, 12 preparations (every 2 months) were reported as being analyzed. The study methods section states that the feed was prepared "twice monthly." The feed prepared on December 12, 1985 was shown to have a wide range for homogeneity (-29 to +52 percent of the nominal concentration) for group 3 (20 ppm). This could mean that the diet may have been as low as 14.2 ppm to as high as 30.4 ppm when this diet preparation was used. This deviation is indicated but such deviations were not so frequent so as to compromise the integrity of the study.

## Results

1. Clinical Signs and Reactions - No dose-related signs of clinical or behavioral reactions were evident among the male test groups.

Regarding female rats, the study report (page 41) states that: 007501

"Signs of ill-health including ataxia, ruffled fur, weight loss, reduced activity, stiff gait and hunched posture were seen in both control and treated rats, predominantly before unscheduled death. There was a broadly dose-dependent increase in the incidence of these signs in the female rats of groups 2 (5 ppm), 3 (20 ppm), and 4 (80 ppm) when compared with the female controls. The increased incidence of these signs correlated with the increased mortality in these groups".

The manner of presentation of the data for clinical signs in the study report does not easily allow TB-I to estimate how many rats were affected with each condition or to assess the degree of the condition without a time-consuming retabulation of the data. The conclusion of the study report that at 5 ppm (0.4 mg/kg/day) females develop behavioral reactions is being accepted by TB_I.

NOEL (for clinical signs) < 5 ppm for females; > 80 ppm for males. Various generalized signs were evident in females.

2. Survival/Mortality - No effect on survival was evident in the males. Survival rates were 70, 77, 68, and 80 percent for the control, low-, mid-, and high-dose male test groups, respectively.

All dosed groups among the females had more unscheduled deaths (fewer survivors) than the control group as indicated in the following table.

| | Unscheduled Deaths[1] | Pituitary Tumor[2] |
|---|---|---|
| Control | 19 (32%) | 8/19 (42%) |
| 5 ppm | 29 (49%)* | 17/29 (59%) |
| 20 ppm | 38 (66%)*** | 24/38 (63%) |
| 80 ppm | 47 (78%)*** | 34/47 (72%) |

* P < 0.05, *** P < 0.001. Fisher's Exact P, HED Computer.

[1]Deaths out of 60 rats scheduled for 104 weeks of dosing, except for 59 in group 2 and 58 in group 3. In these groups rats died following trauma during blood sampling. The 10 rats in the interim sacrifice group are not included in the above calculation.

[2]Pathological analysis of the cause of death indicated pituitary tumors were probably involved in the unscheduled death. This column indicates the number of rats for which the study report maintains that the pituitary tumor was probably fatal (numerator) and the number of unscheduled deaths among rats scheduled for 104 weeks of treatment. These data are presented without a statistical analysis.

NOEL < 5 ppm for deaths among the females.

3. Body Weight, Food and Water Consumption - The NOEL for this aspect of the study is 5 ppm. At 20 ppm, both males and females gained less weight, an effect that was noticeable after the first year. Final body weights were -7.7 percent (statistically significant $p < 0.01$) for males and -4.4 percent (not significant) for females less than controls for the mid-dose (20 ppm) group. Final body weights for the high-dose group were -21.1 percent for males and -16.7 percent for the females; lower body weights were evident throughout most of the study for this group. Decreased food intake was evident for both males and females in the high-dose group only.

4. Ophthalmoscopy - The following table illustrates the results of the ophthalmoscopy examination at study termination (104 weeks).

| | Unilateral and Bilateral Corneal Opacity | |
|---|---|---|
| Dose Group | Males | Females |
| Control | 1/6 | 0/6 |
| 5 ppm | 2/6 | 0/2 |
| 20 ppm | 4/5 | 2/4 |
| 80 ppm | 5/6 | No survivors available |

No evidence of treatment related corneal opacity was apparent in the rats at the 26-, 52-, and 78-week examinations. The study report asserts that no clear relationship exists between severity and dose of TPTH at week 104.

In addition to corneal opacity, the only rats showing "posterior cataract" were rats dosed with TPTH. There were 0/6, 3/6, 1/5, and 1/6 males in the control, low mid and high dose test groups.

The small number of rats examined at week 104 limits the usefulness of the assessment at 104 weeks. The indications of an effect as were noted in this study should have been further evaluated by ophthalmoscopic examination of all other available rats before any rats were sacrificed.

CONCLUSION (Ophthalmoscopy). There were no effects at weeks 26, 52 and 78 weeks. There was an apparent dose response for incidence of opacity without a dose related increase in severity in opacity at week 104. These data do not provide conclusive evidence that TPTH affects the eye in this study.

[Note: For sections 5 and 6 below, blood samples were taken after weeks 26, 52, 78, and 104 from the rats in the chronic feeding aspects of the study. The rats were fasted for 18 hours before sacrifice. Samples were taken in the early morning under light anesthesia from the retro-orbital sinus.

5. Hematology - Parameters investigated included: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count, nucleated erythrocytes-normoblasts, Heinz bodies, total leukocyte count (WBC), differential leukocyte count, red cell morphology, and coagulation (thromboplastin time, partial thromboplastin time).

   Of these parameters, there were occasional significantly differences without dose responses or consistency over the dosing periods. The following showed indications of possible effects of TPTH treatment.

   a. Hemoglobin and Hematocrit - Females showed slight decreases (approximately 5%) at all samplings for the mid- and high-dose groups. The low-dose group also had decreased hemoglobin (-11.5%) at week 104 but the mid-dose group was only -7 percent at this time.

   b. Prothrombin time was decreased (to -13%) for the mid- and high-dose groups but partial thromblastin time was not affected.

   NOEL > 80 ppm for hematology. The differences reported are not considered of sufficient magnitude, consistency or supported by ancillary effects to conclude that they were the results of TPTH dosing.

6. Clinical Biochemistry - The following parameters were investigated: Glucose, urea, creatinine, total and direct bilirubin, total cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase, creatinine kinase, alkaline phosphatase (ALP), gamma-glutamyl-transferase, ornithine carbamyl-transferase, $Ca^{++}$, phosphorous, $Na^{+}$, $K^{+}$, $Cl^{-}$, and protein (total and electrophoresis).

   At 80 ppm, there is evidence of increased serum enzyme activity for three enzymes (ASAT, 12% males, 27% females; ALAT, 18% males and 12% females; and ALP, 46% females only). ASAT was also 20 percent increased for the mid- (20 ppm) dose group females. The NOEL is based on the increase in three enzymes which suggests organ (probably liver) damage.

   $Ca^{++}$ levels were decreased to -6 percent for high-dose group males at week 78 and other groups were up to -5 percent decreased. The $Ca^{++}$ levels, however, were reported as being in the normal range for historical control. [Note: Slightly (-3%) decreased Ca++ levels were also evident for the mid and high dose groups in the subchronic range finding study.]

   Total protein was decreased for the high dose group males at weeks 78 (-6%) and 104 (-8.5%) but no corresponding decreases in the electrophoresis bands were evident. There was no pattern in protein or electrophoresis changes evident in the females to suggest an effect of TPTH.

CONCLUSION (Clinical chemistry): NOEL: 20 ppm. At 80 ppm there were increases in serum enzyme levels (ASAT, ALAT, and ALP). Ca++ and protein level changes were not considered biologically significant.

7. Special Assessment of Immunoglobulins [ANAWA Laboratories, Wanger, Switzerland]

   [Note: TPTH is under investigation as a possible immunotoxin. The rat 90-day subchronic study which was conducted to determine the dose levels for this chronic feeding/oncogenicity study was reviewed previously by TB and it was determined that the study did not show a NOEL for possible effects on immunoglobulin levels (refer to J. Doherty review dated July 18, 1986]. In particular the IgG levels were decreased at all dose levels for females after the recovery period.

   Immunoglobulin assessments were made at weeks 50 and 81 using all of the rats from the chronic feeding aspect

of the study and from the interim sacrifice group at week 50. The following immunoglobulins were assessed: G1, G2a, G2b, G2c, A, and M. Assessments were made with antisera and laser nephelometry (PEG enhanced).

Statistically significant decreases in immunoglobulins were not evident at 81 weeks. There were occasions of increases (IgG2b, 44% high-dose males, IgG2c 63% low-dose females) but these did not suggest an effect of TPTH.

At 50 weeks, there were numerous decreases and occasional increases as indicated in the following summary. [The IgG levels did not show decreases for either sex but the subgroups of the IgG immunoglobulins apparently were affected.

| | Sex | Dose Group | | |
|---|---|---|---|---|
| Immunoglobulin | | Low | Mid | High |
| IgG1 | F | -39%* | -57%* | -50%* |
| | M | +12% NS | +52% NS | +83%* |
| IgG2a | M | - | -26%* | -17% NS |
| | F | -21%* | -36%* | -49%* |
| IgG2c | M | -37%* | -46%* | -34%* |
| | F | -30%* | -16% NS | -11% NS |
| IgA | M | -9% NS | -38%* | -42%* |
| | F | -4% NS | -22% NS | -35% NS |
| IgM | M | -23% NS | +61%* | +62%* |
| | F | +20% NS | +42%* | +52%* |

Data are presented as percent difference from the control and are statistically significant unless otherwise indicated by NS. The - means that the set was equivalent to the control.

CONCLUSION (Immunoglobulins): Decreases in immunoglobulins are indicated at all levels (NOEL < 5 ppm).

8. Urinalysis - Samples of urine were collected following an 18-hour fast (deprivation of food but not water) at weeks 26, 52, 78, and 104. The following parameters were assessed: volume, specific gravity, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, and urine sediment.

NOEL (urinalysis) = 80 ppm.

9. Organ Weights - The following organs were weighed at the 1-year interim and terminal sacrifice periods: adrenals, brain, heart, kidneys, liver, spleen, pituitary gland, ovaries, testes, and thyroid gland. Organ to body weight and organ to brain weight ratios were determined.

   The study report asserts that no changes in organ weights were of toxicological significance and the changes noted were probably attributable to the differences in terminal body weights.

The following organs showed weight changes that were noted by TB-I as possibly being related to TPTH dosing after 104 weeks of dosing.

a. Liver - Males - Absolute weights for the mid (-12.2%) and high (-24.2%) dose groups were reduced. Liver to body weight ratios were not affected although body weights were reduced. Liver to brain weight ratios for the mid (-13%) and high (-25%) dose groups were also reduced. No effects were apparent on female liver weights or ratios. The rather marked dose response and consistency between absolute and brain weight ratios suggest a possible effect in the males but this decrease may be related to the generalized body weight decrease. The evidence for increased serum enzyme activity (ASAT, ALAT, and ALP) provide some indication of a possible effect of TPTH in the liver. Refer to Section 11c below for the conclusions regarding the liver.

b. Heart - Males - The mid (-8%) and high (-14.5%) dose groups were decreased for absolute weight. The high dose group was increased (+12%) relative to body weight and both the mid (-9%) and high (-16%) dose levels the heart to brain ratio was decreased. Female heart weights in the high dose group were elevated (+13%) for the body weight ratio only. These data suggest that the heart of males may be affected by TPTH dosing. Refer to Section 11e below for conclusions regarding the heart.

c. Spleen - [Note: The spleen is regarded as a possible target organ for TPTH because of its possible immunotoxicity.] Spleen/body weight ratios for males were elevated at termination (+20%) and at 52 weeks (17%) for the high dose

group. Female spleen weights were equivalent to the controls. See also Section 11d below.

d. Testis - The testis weight relative to body weight was elevated (+27%) for the high dose group but the absolute weight and weight relative to brain weight were not statistically increased. Refer to Section 11b below.

e. Statistical differences in the absolute weights or ratios for the brain, testes, and kidneys were not considered by TB-I to be related to TPTH dosing.

CONCLUSION (Organ weights): NOEL = 5.0 ppm. At 20 ppm and above, liver and heart weight decreases in males are apparent. [Note: There were no statistical differences in pituitary weights.]

10. Macroscopic Pathology - Evidence of increased incidence of "pituitary gland nodules" associated with "compression of the brain" were evident at necropsy in the females but not males. Refer to Section 11a below for discussion. No other dose-related abnormalities were evident.

11. Histopathology - [The pathology report was signed by Drs. Burkhard Schlotke and Hans-Joerg Chevalier and the work was done at Experimental Pathology Services, AG Hauptstrasse, Switzerland).

The survivors were sacrificed by intraperitoneal injection of sodium pentobarbital and exsanguination. A comprehensive list of 44 tissues/organs were dissected out and prepared for histopathology. A comprehensive microscopic examination was reported as being made for all rats in the control and high-dose groups. Microscopic evaluation of the low- and mid-dose groups was limited to the brain, heart, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), pituitary gland, sciatic nerve, skeletal muscle, spinal cord (cervical, mid-thoracic, lumbar), spleen, testes, thymus, and all gross lesions.

The following is a topical organ discussion of the histopathology findings.

a. Pituitary - The following table presents the neoplastic and non-neoplastic findings for the pituitary (including associated brain) gland and pars intermedia.

| Lesion | | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|---|
| Macroscopic | N | | 70/70 | 70/70 | 70/70 | 70/70 |
| Nodules | | M | 17 | 16 | 21 | 18 |
| | | F | 21 | 26 | 33 | 47 |
| Compressing Brain | | M | 11 | 11 | 14 | 8 |
| | | F | 15 | 15 | 26 | 31 |
| Microscopic | | | | | | |
| Pars Intermedia | N | | 53/49 | 55/41 | 56/36 | 57/46 |
| hyperplasia | | M | -- | -- | -- | 12*** |
| cystoid change | | M | 8 | 11 | 29*** | 38*** |
| | | F | 10 | 8 | 6 | 28*** |
| Neoplastic Pituitary Gland | N | | 68/69 | 68/67 | 67/66 | 69/68 |
| Adenoma (Total) | | M | 24 | 20 | 37 | 26 |
| | | F | 39 | 37 | 43 | 56*** |
| Adenoma (among decedents) | N | | 19/19 | 13/29 | 19/38 | 13/46 |
| | | M | 10 | 7 | 12 | 10 |
| | | F | 14 | 20 | 33 | 43 |

*** p < .001. Fisher's Exact P, HED Computer.

The above table indicates that the pituitary in both the male (non-neoplastic) and female (neoplastic and non-neoplastic) is a target organ for dietary TPTH.

The pituitary pars intermedia of the male demonstrated increased incidence of hyperplasia, an uncommon lesion in this strain of rat (high-dose group only) and cystoid change (15.1, 20.0, 51.8, and 66.6 percent for the control, low-, mid-, and high-dose groups) at possibly all test dose groups. Evidence of increased incidence of cystoid change was apparent in both the mid- (33.3%) and high- (40%) dose group males at the 52-week sacrifice interval (control: 14.3%). The mid-dose male group had a higher incidence of adenomas than the control (55.2 vs. 35.3%). The historical control data for this strain of rat (Wistar, KFM-Han,

appended) indicates that the maximum percentage incidence for pituitary adenomas in males for 13 studies is 54%. The 55.2% incidence for the mid dose group is sightly larger than the 54% range limit, the high-dose group incidence (44.1%), however, is equivalent to the controls and within the historical control range. Thus the pituitary gland of males need not be regarded as an oncogenic target organ for TPTH.

Indications that the pituitary is a target organ in females for TPTH were evident at necropsy since 30, 37.1, 47.1, and 67.1 percent of the females in the control, low-, mid-, and high-dose groups had visible nodules. Evidence that enlarged pituitaries were compressing the brain was apparent in the mid (37.1% incidence) and high (44.3%) dose groups because fewer (21.4%) rats were affected in both the control and low-dose groups.

As indicated in the table above, the incidence of non-neoplastic lesion of cystoid change in the pars intermedia was increased in the high-dose group females (60.9 vs. 20.4% for the controls). At the 1-year interim sacrifice, 8 of 10 females (80%) in the high dose group but none in the control had this condition indicating early (< 1 year) effects of TPTH in the pituitary.

An oncogenic effect of TPTH in the pituitary is indicated by the dose related progression of 56.5, 55.2, 65.2, and 82.4 percent of the females having adenomas in the control, low-, mid-, and high-dose test groups. Note: When only the rats scheduled for 104 weeks of dosing are considered the % incidence in the high dose group is 93%. Historical control data provided by the RCC Laboratory (appended) indicated that the range for spontaneous occurrence of pituitary adenomas in females is 40-79% for 13 studies conducted in 1985-1988. The high dose female group (82.4 to 93% incidence) is clearly in excess of the historical control range.

There were no malignant tumors in the pituitary.

A statistical analysis of adenoma data in the females including the interim sacrifice groups and adjustments for survival will be prepared by HED and presented separately.

CONCLUSION (Pituitary): NOEL = 5 ppm. At 20 ppm there is cystoid change in males. At 80 ppm these is cystoid change in

males and females and hyperplasia in males. Pituitary adenomas are statistically increased in the high dose group.

b. Testes - The following table illustrates the microscopic findings in the testes.

| Lesion | Test Group Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|
| N | 60 | 59 | 60 | 60 |
| Non-neoplastic | | | | |
| Leydig Cell Hyperplasia | 5 | 6 | 11 | 24***(1) |
| Tubular Atrophy | 6 | 9 | 10(1) | 17** |
| Neoplastic | | | | |
| Leydig Cell Tumors | 1 | 5 | 3 | 10**(1) |

** P < 0.01 and *** P < 0.001. Fisher's Exact P, HED computer. The numbers in () represent the incidence at 52 weeks and is not included in the statistics.

The above table shows that there are at least two types of non-neoplastic pathological changes showing dose-related increases in incidence: The above table also indicates that the testes is an oncogenic target organ for TPTH since there were 1.67, 8.48, 5, and 16.7 percent incidence of Leydig cell tumors for the control, low-, mid-, and high-dose test groups based on 60 rats dosed for 104 weeks. There was also one rat affected with a Leydig Cell tumor in the high dose interim sacrifice group. Thus there were a total of 15.7% rats in the high dose group affected with this tumor type.

This type of tumor was reported to have a range in percentage occurrence of 0-8% for twelve studies and a 13th study had 16% incidence based on historical control information provided by the RCC laboratory (appended). The combination of there being dose related non-neoplastic and neoplastic pathology in the testis lessens the probability that the high rate of tumors in the high dose group is due to chance alone. Inspection of the individual animal data revealed that of the 20 rats affected with Leydig Cell tumors only a single rat (mid dose group) did not also have either hyperplasia or tubular atrophy. Six rats had the tumor

and atrophy, five rats had the tumor and hyperplasia, and eight rats had all three lesions.

There were no malignant tumors in the testis.

CONCLUSION (Testis): NOEL = 20 ppm. LEL = 80 ppm, Leydig Cell hyperplasia and tubular atrophy. Leydig Cell tumors are increased in high dose group.

A statistical analysis of the Leydig cell tumor data will be prepared by HED and presented separately.

c. Liver - The liver showed weight decreases and serum enzyme activity was elevated indicative of possible liver injury. The following table summarizes some of the histopathological findings in the liver.

| Lesion | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| N | | 60/59 | 60/59 | 60/58 | 60/58 |
| Bile Duct Proliferation | F | 23 | 20 | 34* | 44*** |
| Portal Sclerosis | F | 16 | 16 | 32** | 49*** |
| Eosinophilic focus | M | 2 | 2 | 6 | 19*** |
| Hepatocellular Carcinoma | M | 1 | 0 | 1 | 0 |
| | F | 1 | 0 | 0 | 0 |
| Hepatocellular Adenoma | M | 0 | 0 | 0 | 0 |
| | F | 0 | 1 | 1 | 0 |

* P < 0.05, ** P < 0.01, *** P < 0.001. Fisher's Exact P HED Computer.

The above table indicates that there are dose-related increased incidences of bile duct proliferation and portal sclerosis in the females at the mid- and high-dose test groups. These two lesions were also noted to be increased in the liver at the 1-year interim sacrifice with there being a higher incidence in the high-dose group (56%) than in the control (10%) for bile duct hyperplasia. There was 0, 10, 20 and 60% incidence of portal sclerosis for the control, low, mid and high dose groups at interim sacrifice.

The liver tumor data (hepatocellular carcinoma and

adenoma) are shown in the above table because liver tumors were noted to be increased in response to TPTH treatment in the mouse oncogenicity study (RCC Study No. 047002, April 14, 1989). As indicated by the above table, there is no indication that the liver is an oncogenic target organ for TPTH.

CONCLUSION. NOEL (liver) = 5 ppm. LEL = 20 ppm for increases in bile duct proliferation and portal sclerosis. LEL = 80 ppm for increases in eosinophilic focus. The liver weight and serum enzymes changes are supported by histopathological changes in the liver.

d. Spleen - The spleen is a part of the immune system and immunotoxic agents often affect the histopathology of this organ and at termination of the study spleen weight for males was increased. There were no indications of dose-related increases in histopathological findings in this organ.

e. Heart - Heart weights for males were elevated slightly at termination of the study. The incidence of myocardial fibrosis was higher in the high-dose group males (47/60 or 78%) than in the controls (36/60 or 60%), low (30/60 or 50%) and mid (38/59 or 64%). For females there was a negative progression for myocardial fibrosis (27.1%, 8.5%, 10.2% and 5.2%) for the control, low, mid and high dose groups. Note: Fibrosis would be expected to result in an increase in heart weight.

In CONCLUSION (heart), myocardial fibrosis is regarded as a lesion type that varies widely. Thus, the heart weight changes (decreases) are not supported by pathological changes in the heart (at least at the low and mid dose groups). The weight change may be related to the generalized body weight decrease. TB-I does not consider that the heart is a target organ for TPTH based on the data in this study.

f. Skeletal Muscle - Increased incidence of atrophy in the skeletal muscle was noted as indicated in the following table.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| | N | 60/60 | 59/58 | 60/57 | 60/57 |
| Atrophy | M | 1 | $5^{NS}$ | 9** | 14*** |
| | F | 0 | 0 | 0 | $3^{NS}$ |

** P < 0.01, *** P < 0.001. Fisher's Exact P. HED Computer.

It is apparent that all dose levels among the male groups and the high-dose female group show some increased incidence of skeletal muscle atrophy.

CONCLUSION (skeletal muscle). NOEL = 5 ppm. LEL 20 ppm males have increased incidence of skeletal muscle atrophy. Females may be affected at 80 ppm or higher.

g. Sciatic Nerve - There was evidence that the mid- (55.9% or 33/59) and high- (51.7% or 31/60) dose male groups had increased incidence of degenerative neuropathy in the sciatic nerve when compared with the control (33.3% or 20/60) and low-dose group (33.9% or 20/59). The statistical significance of the trend was $p < 0.05$ (study report analysis). At 52 weeks the only incidence of degenerative neuropathy was in the high dose male group. Among the females the control group had 16.7% incidence and the high dose had 24.1% incidence. The low and mid dose groups had 15 and 6.9% incidence respectively.

TENTATIVE CONCLUSION (sciatic nerve). NOEL = 5 ppm. LEL = 20 ppm males. The study data demonstrate that there are increased incidences of sciatic nerve degeneration.

NOTE. If the registrant produces historical control data for the strain of rat used in this study and demonstrates that the mid and high dose group incidence is within the range for historical control incidence for degeneration of the sciatic nerve, TB-I will reconsider the assignment of a NOEL of 5 ppm for this lesion.

h. Brain - The evidence of compressed areas indicated at necropsy was confirmed microscopically. Among the female groups there were 25, 32, 48, and 64 percent incidence for rats with "area of compression" for the control, low-, mid-, and high-dose groups, respectively.

Note: The organotin chemicals, particularly the ethyl and methyl tins, affect the nervous system by causing swelling and consequential damage. There was no evidence of such lesions presented in this study for TPTH.

i. Kidney - Many xenobiotics specifically affect the kidney of male rats. The telltale signs for this effect include tubular degeneration, swelling, hyaline droplet formation, and mineralization.

None of these lesion types were increased in a dose dependent manner in response to TPTH in this study. No other apparent effects of TPTH were evident in the kidney.

J. Adrenals. The adrenals were identified as a target organ in the rat (medullary pheochromocytomas) in a study with tributyltin oxide a structural analog of TPTH (WHO study, February, 1988, study not formally reviewed by TB). In the current study with TPTH there were 2 incidences of cortical carcinomas (one female low dose group and one male mid dose group) and three incidence of cortical adenomas (one female control and two female mid dose group). There were no pheochromocytomas reported.

K. Thyroid. The pathological data for the pituitary and testis might possibility be related to pathological changes in the thyroid. There were no dose related increases in non-neoplastic or neoplastic lesions in the thyroid. Non-neoplastic lesions were cystic like (up to about 3 per dose group) or hyperplasia (control male group had the highest incidence, 16.7%). The control group males (10.7%) and control females (15%) had the highest incidence of C cell adenomas. Since thyroid weights were not affected there is no evidence that TPTH affected the thyroid in this study.

12. Maximum Tolerated Dose (MTD) Considerations.

The MTD is considered to have been met based at least upon body weight decreases. Whether or not the MTD was exceeded to the extent that there was competing toxicity to compromise the interpretation of the neoplastic findings in both the pituitary and testis will be discussed during the oncogenicity Peer Review meeting for TPTH.

Conclusion:

This study is CORE-GUIDELINES. The study did not establish a NOEL. The following effects of TPTH were noted:

Neoplastic. The pituitary (females) and testis have been identified as target organs for TPTH in the rat. This information will be Peer Reviewed by HED for carcinogenicity classification of TPTH.

Systemic effects.

- NOEL < 5 ppm (0.4 mg/kg females). At this level there were increases in deaths and behavioral reactions in females probably associated with pituitary tumors. Decreases in immunoglobulins (IgG1, Ig2a, Ig2c, IgA).

- LEL = 20 ppm, decreases in body weight gain; decreases in liver weight; "cystoid change" in pituitary (males); nodules (pituitary, females) and compression of brain; liver bile duct proliferation and portal sclerosis. Skeletal muscle atrophy in males and degenerative neuropathy in the sciatic nerve (males, tentative conclusion, additional historical control data requested).

- LEL = 80 ppm, decreases in food consumption, increases in serum enzyme activity (ASAT, ALP, and ALAT), pituitary pars intermedia hyperplasia (males), Leydid Cell hyperplasia and testicular tubular atrophy, liver eosinophilic focus (females).

HISTORICAL CONTROL TUMOR INCIDENCE
WISTAR RAT

007501

ORGAN: PITUITARY GLAND

ADENOMA

| PROJECT | STUDY TYPE | REPORT | PATH. | ANIMALS EXAMINED | | ANIMALS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 005321 | 72 WEEK FEEDING | 1985 | JMA | 48 | 50 | 9 | 26 | 19 | 52 |
| 005321 + | 72 WEEK FEEDING | 1985 | JMA | 49 | 50 | 20 | 20 | 41 | 40 |
| 006390 | 72 WEEK FEEDING | 1985 | RUD | 49 | 48 | 7 | 24 | 14 | 50 |
| 006390 + | 72 WEEK FEEDING | 1985 | RUD | 49 | 49 | 15 | 24 | 31 | 50 |
| 017820 | 104 WEEK FEEDING | 1986 | HHW | 50 | 50 | 20 | 29 | 40 | 58 |
| 024300 | 104 WEEK FEEDING | 1988 | JMA | 100 | 99 | 51 | 73 | 51 | 74 |
| 027753 | 104 WEEK I. M. | 1988 | JMA | 51 | 51 | 19 | 31 | 37 | 61 |
| 008831 | 116 WEEK FEEDING | 1986 | PAG | 48 | 49 | 26 | 36 | 54 | 73 |
| 027472 | 120 WEEK FEEDING | 1987 | BSC | 49 | 50 | 17 | 39 | 35 | 78 |
| 004285 | 130 WEEK FEEDING | 1985 | BSC | 48 | 48 | 26 | 38 | 54 | 79 |
| 014387 | 130 WEEK FEEDING | 1986 | JAW | 49 | 49 | 8 | 25 | 16 | 51 |
| 014387 + | 130 WEEK FEEDING | 1986 | JAW | 49 | 49 | 11 | 27 | 22 | 55 |
| 018505 | 130 WEEK FEEDING | 1986 | JAW | 48 | 49 | 18 | 25 | 52 | 51 |

PARS INTERMEDIA ADENOMA

| PROJECT | STUDY TYPE | REPORT | PATH. | ANIMALS EXAMINED | | ANIMALS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 005321 | 72 WEEK FEEDING | 1985 | JMA | 48 | 50 | - | - | 0 | 0 |
| 005321 + | 72 WEEK FEEDING | 1985 | JMA | 49 | 50 | - | - | 0 | 0 |
| 006390 | 72 WEEK FEEDING | 1985 | RUD | 49 | 48 | - | - | 0 | 0 |
| 006390 + | 72 WEEK FEEDING | 1985 | RUD | 49 | 49 | - | - | 0 | 0 |
| 017820 | 104 WEEK FEEDING | 1986 | HHW | 50 | 50 | - | 1 | 0 | 2 |
| 024300 | 104 WEEK FEEDING | 1988 | JMA | 100 | 99 | - | - | 0 | 0 |
| 027753 | 104 WEEK I. M. | 1988 | JMA | 51 | 51 | - | - | 0 | 0 |
| 008831 | 116 WEEK FEEDING | 1986 | PAG | 48 | 49 | - | - | 0 | 0 |
| 027472 | 120 WEEK FEEDING | 1987 | BSC | 49 | 50 | - | - | 0 | 0 |
| 004285 | 130 WEEK FEEDING | 1985 | BSC | 48 | 48 | - | - | 0 | 0 |
| 014387 | 130 WEEK FEEDING | 1986 | JAW | 49 | 49 | - | - | 0 | 0 |
| 014387 + | 130 WEEK FEEDING | 1986 | JAW | 48 | 49 | - | - | 0 | 0 |
| 018505 | 130 WEEK FEEDING | 1986 | JAW | 48 | 49 | - | - | 0 | 0 |

+ 2nd control group.

HISTORICAL CONTROL TUMOR INCIDENCE
WISTAR RAT



TESTES

LEYDIG CELL TUMOR

| PROJECT | STUDY TYPE | REPORT | PATH. | ANIMALS EXAMINED | | ANIMALS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 005321 | 72 WEEK FEEDING | 1985 | JMA | 50 | | - | | 0 | |
| 005321 + | 72 WEEK FEEDING | 1985 | JMA | 49 | | - | | 0 | |
| 006390 | 72 WEEK FEEDING | 1985 | RUD | 50 | | 3 | | 6 | |
| 006390 + | 72 WEEK FEEDING | 1985 | RUD | 50 | | 2 | | 4 | |
| 017820 | 104 WEEK FEEDING | 1986 | HHW | 50 | | 1 | | 2 | |
| 024300 | 104 WEEK FEEDING | 1988 | JMA | 100 | | - | | 0 | |
| 027753 | 104 WEEK I. M. | 1988 | JMA | 52 | | 1 | | 2 | |
| 008831 | 116 WEEK FEEDING | 1986 | PAG | 50 | | 1 | | 2 | |
| 027472 | 120 WEEK FEEDING | 1987 | BSC | 49 | | 4 | | 8 | |
| 004285 | 130 WEEK FEEDING | 1985 | BSC | 50 | | 4 | | 8 | |
| 014387 | 130 WEEK FEEDING | 1986 | JAW | 50 | | 2 | | 4 | |
| 014387 + | 130 WEEK FEEDING | 1986 | JAW | 49 | | 8 | | 16 | |
| 018505 | 130 WEEK FEEDING | 1986 | JAW | 50 | | 1 | | 2 | |

MESOTHELIOMA

| PROJECT | STUDY TYPE | REPORT | PATH. | ANIMALS EXAMINED | | ANIMALS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 005321 | 72 WEEK FEEDING | 1985 | JMA | 50 | | - | | 0 | |
| 005321 + | 72 WEEK FEEDING | 1985 | JMA | 49 | | - | | 0 | |
| 006390 | 72 WEEK FEEDING | 1985 | RUD | 50 | | - | | 0 | |
| 006390 + | 72 WEEK FEEDING | 1985 | RUD | 50 | | - | | 0 | |
| 017820 | 104 WEEK FEEDING | 1986 | HHW | 50 | | - | | 0 | |
| 024300 | 104 WEEK FEEDING | 1988 | JMA | 100 | | - | | 0 | |
| 027753 | 104 WEEK I. M. | 1988 | JMA | 52 | | 1 | | 2 | |
| 008831 | 116 WEEK FEEDING | 1986 | PAG | 50 | | - | | 0 | |
| 027472 | 120 WEEK FEEDING | 1987 | BSC | 49 | | - | | 0 | |
| 004285 | 130 WEEK FEEDING | 1985 | BSC | 50 | | - | | 0 | |
| 014387 | 130 WEEK FEEDING | 1986 | JAW | 50 | | 1 | | 2 | |
| 014387 + | 130 WEEK FEEDING | 1986 | JAW | 49 | | - | | 0 | |
| 018505 | 130 WEEK FEEDING | 1986 | JAW | 50 | | - | | 0 | |

+ 2nd control group.

007501

Reviewed By: John Doherty 9/20/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl Baetcke 9/21/89
Chief, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-2 - Oncogenicity - Mice

MRID No.: 410857-01 (five volumes) TOX Chem No.: 896E

Test Material: Technical grade triphenyltin hydroxide, 97.2% pure, Code HOE 029664 of ZD97 0004, described as a powder.

Test Animals: NMRI mice KFD-HAN (Specific Pathogen Free), obtained from KFM Kleintierfarm Madorin AG, Switzerland. They were about 5 weeks of age at the start of dosing. They were housed individually.

Synonyms: TPTH, fentin hydroxid

Study No.: 047002

Sponsor: Hoeschst Celanese Corporation
Somerville, New Jersey

Testing Facility: Research & Consulting Company (RCC), Itingen, Switzerland. Specialized assessments were made at other laboratories, refer to DER text.

Title of Report: TPTH Technical (Code HOE 029666 of ZD97 0004) Oncogenicity 80 Week Study in Mice.

Authors: H. Tennekes, K. Horst, H. Luetkemeier, W. Vogel, O. Vogel, J. Armstrong, H.A. Bhiers, E. Muller, Ch. Terrier.

Report Issued: April 14, 1989

Conclusions:

This study is classified CORE GUIDELINE.

Oncogenicity findings: This study demonstrates evidence of oncogenicity based on increased incidence of liver adenomas (males and females) and carcinomas (females).

Systemic findings: At 5 ppm and above there were decreases

in immunoglobulins.

At 20 ppm and above: increases in body weight (females, first months of study), kidney weight decreases (without pathological changes). Skin lesions.

At 80 ppm: deaths and changes in general appearance (females); decreased weight gain and liver weight increases.

[Note: No NOEL and LEL are assigned since this study is not a chronic feeding study.]

Special Review Criteria (40 CFR 154.7):

Evidence of oncogenicity meets the criteria for Special Review.

Quality Assurance Statement:

A statement was provided which was signed for the Quality Assurance Manager (K. Schneider) by a individual whose signature was illegible. The statement indicated that 10 reports of Quality Assurance inspections were made. These inspections did not include the immunoglobulin assessments which were performed at the ANAWA Laboratories.

Review

The basic study design consisted of four groups of 50 male and female mice which were dosed with 0, 5, 20, and 80 ppm of TPTH for 80 weeks (approximately 18 months). The selection of these dose levels was based on a 90-day subchronic dose range-finding study (refer to review by J. Doherty dated July 15, 1986). It should be noted here that this range-finding study was not considered by TB-I to demonstrate a NOEL with regard to effects of TPTH on immunoglobulin levels.

Analysis of the feed, body weight, and food consumption indicated that the dose levels for each group in mg/kg/day of TPTH were as follows:

Dose Levels (mg/kg/day)

| | Males | Females |
|---|---|---|
| Control | 0 | 0 |
| 5 ppm | 0.85 | 1.36 |
| 20 ppm | 3.50 | 4.56 |
| 80 ppm | 15.24 | 20.16 |

Results

1. Mortality/Survival - The following table illustrates the number of spontaneous deaths/survivors in this study.

| | Death/Survival[1] Males | Females |
|---|---|---|
| Control | 10/39(1) | 15/32/(3) |
| 5 ppm | 8/41(1) | 19/26(5) |
| 20 ppm | 16/34 | 16/30(4) |
| 80 ppm | 16/34 | 26/24* |

* P < 0.05 Fishers Exact P, HED Computer.

[1]The number in () is the number of mice that died accidentally following blood sampling. These mice were included in the histopathology examination. The high-dose female group had a

significant greater number of spontaneous deaths. The difference in survival in the high-dose group females was apparent after the 50th week of the study.

NOEL = 20 ppm, LEL = 80 ppm for deaths (females).

2. Clinical Signs - The high-dose group females were reported to have signs of "ill health" including "ruffled fur" toward the end of the study. The male mice and the females in the lower dose groups were reported as being similar to the controls.

   NOEL = 20 ppm, LEL = 80 ppm for changes in appearance in females.

3. Body Weight and Food Consumption - The high-dose group males gained less weight than the controls from the beginning of the study and until termination when their body weight was 12 percent less than the controls.

   The body weights for the females were at first higher up to approximately week 49 for the mid-dose group (about 6-9%) and week 41 for the high-dose group (about 10-12%). After week 65, the high-dose group females were lower in body weight until at termination they were 15 percent less.

   The food consumption pattern did not reflect the changes in body weight since only an increase in the high-dose male group was reported.

   NOEL = 5 ppm, LEL = 20 ppm, females have increased weight gain. LEL = 80 ppm males and females (after week 65) for decreased weight gain.

4. Ophthalmoscopy - Ophthalmoscopic examinations were performed at months 6, 12, and 18. No test chemical-related effects were evident. Note: Some possible effects in the eye were noted in the rat chronic feeding study (RCC Study No. 046980, April 18, 1989).

5. Hematology - Blood samples were collected at week 80 from 10 mice per sex per dose group. The sample was taken from fasted mice under light ether anesthesia. The parameters investigated included: nucleated erythrocytes, normoblasts, differential leukocyte count (including cell classification) and red cell morphology.

   There were no compound-related effects on these

parameters.

6. Special Assessment of Immunoglobulins - Blood samples were reportedly collected from all available mice prior to necropsy (over a two week span) and the following immunoglobulins were quantitated: G, G1, G2a, G2b, G3, A, and M. The immunoglobulins were assessed using antiserum and laser nephelometry (PEG enhanced). The following table illustrates the findings showing effects of TPTH treatment on the various immunoglobulins.

| | | Dose Level[1/] | | |
|---|---|---|---|---|
| Immunoglobulin | Sex | 5 ppm | 20 ppm | 80 ppm |
| IgG | M | 92 | 95 | 66* |
| | F | 87 | 95 | 46* |
| IgA | M | 69* | 82 | 72* |
| | F | 77* | 80* | 76* |
| IgM | M | 78* | 70* | 60* |
| | F | 98 | 101 | 73* |
| IgG2b | M | 100 | 74* | 64* |
| | F | 120 | 95 | 63* |
| IgG3 | M | 91 | 74* | 81* |
| | F | 112 | 93 | 61* |
| IgG1 | F | 107 | 98 | 61* |
| IgG2a | F | 103 | 83 | 50* |

[1]Data are in percent of control value.
*Statistically significantly different from the control group as determined by the testing laboratory.

The above table indicates that there is no NOEL for decreases in the levels of IgA (males and females) and IgM (males). A LEL of 20 ppm is noted for IgG3 (males), and Ig2b (males). A LEL of 80 ppm is recognized for IgG (males and females), IgM (females), IG2b (females), IgG3 (females), IgG1 (females), and IgG2a (females).

It should be noted that the 90-day range-finding study demonstrated that effects (decreases) were also evident in IgG, IgA, and IgM in both males and females.

The testing laboratory acknowledges the decreases as above but does not distinguish these effects as a primary effect of TPTH. In particular, the report states that the decreases in IgA levels may result from a local irritant effect of TPTH on mucus membranes.

CONCLUSION (Immunoglobulin assessment). TB-I notes that there are decreases in certain immunoglobulin levels at all dose levels (NOEL < 5 ppm). TB-I, however, considers that this observation must be interpreted with caution. The assessments for immunoglobulins were made only once, there were large standard errors and total serum protein and albumin and globulin were not assessed simultaneously. There were also no pathological correlates associated with the decreases in immunoglobulins. Thus the decreases as above are noted but are considered a possible but not necessarily a definite response to TPTH.

7. Organ Weights - The following organs were weighed at necropsy: adrenals, brain, heart, kidneys, liver with gall-bladder, ovaries, and testes. The data were reported as absolute weight, weight relative to body weight and brain weight. The following table illustrates the changes in organ weights noted.

| Organ | | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|
| Liver Absolute | M | -1/ | - | +24.0%** |
| | F | - | +11.8%NS | +9.9%NS |
| Relative to Body Wgt | M | - | - | +40.3%** |
| | F | - | - | +34.1% |
| Relative to Brain Wgt | M | - | - | +24.1%** |
| | F | - | +7.7%NS | +17.6%** |
| Kidney Absolute | M | - | -7.3%* | -18%** |
| | F | - | -3.3%NS | -20.2%** |
| Relative to Body Wgt | M | -2%NS | -6.2%NS | -8.4%* |
| Relative to Brain Wgt | M | -2%NS | -9.3%** | -18.2%** |
| | F | - | -6.2%NS | -14.4%** |
| Heart Absolute | M | -4%NS | -5%NS | -14%** |
| | F | - | - | -22.1%** |
| Relative to Brain Wgt | M | -4.2% | -7.5%NS | -14.1%** |
| | F | - | - | -16.2%** |
| Brain Absolute | F | - | - | -7.1%** |
| Relative to Body Wgt | M | - | - | +11.5%** |

| | | | |
|---|---|---|---|
| F | +9.3%* | +3.0% | +12.9%** |

1/Organ weight or ratio very close to control and not indicative of an effect similar to the higher dose level.
*Statistically significant $p < .05$.
**Statistically significant $p < .01$
NS Not significant. Data show trend.

The testing laboratory considers that the apparent effects on the heart and brain are coincidental with the decreases in body weight and are not a result of TPTH toxicity. Refer to Sections 9C and 9D below for additional discussion of the heart and brain.

NOEL = 5 ppm; LEL = 20 ppm, kidney weight decreases; LEL = 80 ppm, liver weight increases.

8. Gross Necropsy - Notable findings included increases in liver nodules, foci, and nodular lesions (refer to Section 9a below) and on the skin such as sores and eschars, alopecia occurring on the back/cervical region but also in other regions.

9. Histopathology - The pathology report was presented in Attachment 5 and was prepared (and signed) by James W. Armstrong and Hans-Joerg Chevalier, both veterinary pathologists both affiliated with the Experimental Pathology Services, Hauptstrausse, Switzerland.

Following sacrifice (or spontaneous death), the mice were necropsied and the tissues preserved in 4% neutral phosphate-buffered formaldehyde and imbedded in paraffin, then they were cut into sections of 2 to 4 micrometers and stained with hematoxylin and eosin. A comprehensive sampling of some 44 or more organs/tissues were prepared. All tissues/organs were examined microscopically for the control and high-dose groups. Only the brain, kidneys, liver, lungs, mandibular lymph node, mesenteric lymph node, sciatic nerve, spinal cord, spleen, thymus, and all gross lesions were examined for the low- and mid-dose groups.

The following individual organs are discussed.

a. Liver - Liver to body weight ratios were increased for both sexes for the high dose groups and liver nodules were increased at necropsy. The following table illustrates the pathological findings in the liver.

| Lesion | No.[2/] | Dose Group: Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| | | 50/50 | 50/50 | 50/50 | 50/50 |
| Nodules[1/] | M | 4 | 9 | 8 | 16 |
| | F | 2 | 0 | 1 | 6 |
| Neoplastic Findings | | | | | |
| | No. | 49/50 | 50/50 | 50/50 | 50/50 |
| HEPATO-CELLULAR ADENOMA | M | 6 | 10 | 13 | 16* |
| | F | 0 | 0 | 0 | 9*** |
| HEPATO-CELLULAR CARCINOMA | M | 2 | 1 | 0 | 3 |
| | F | 0 | 0 | 0 | 3 |
| Non-Neoplastic Findings | | | | | |
| NODULAR HYPERPLASIA | M | 2 | 1 | 0 | 5NS |
| | F | 1 | 0 | 1 | 6NS |
| Fatty Change | M | 38 | 43 | 27 | 9 |
| | F | 34 | 32 | 22 | 12 |

* P < 0.05, *** P < 0.001. Fisher's Exact P. HED Computer

1/Lesions written in capital letters show dose response increases.

2/The number of males and females examined is given as males/females.

These data indicate the TPTH treatment results in increased incidence of hepatocellular adenomas in males (possibly all three dose levels) and females (high-dose group) and possibly hepatocellular carcinomas (high-dose group females).

The historical control data provided by the testing laboratory indicate that the range for hepatocellular adenoma is 0-16% in males and 0-4% in females and for hepatocellular carcinoma it is 0-8% in males and 0-2% in females based on 12 studies conducted between 1983 and 1988. In the current study, the high dose female group had 18% incidence of adenomas and 6% incidence of carcinomas clearly in excess of the historical

control. Among the males, the control group (12.2%) was within historical control limits but the low (20%), mid (26%) and high (32%) were all in excess of the historical control range for adenoma. All male groups were within historical control range limits for carcinomas.

The high-dose group males and females also have higher incidence of nodular hyperplasia, a possible preneoplastic condition but statistical significance was not attained.

The incidence of "fatty change" (shown above) and "microgranulomas" (not shown) demonstrated marked decreases as the dose level of TPTH increased.

CONCLUSION (liver): The above data implicate the mouse liver as a target organ for an oncogenic effect of TPTH.

b. Kidney - Kidney weight was decreased for the low- and mid-dose males and high-dose group females.

The following conditions were prevalent in the kidney tissues without regard to increased incidence with increased dose level.

| | Males | Females |
|---|---|---|
| Mononuclear Inflammation | 86-92% | 50-76% |
| Glomerulosclerosis | 94-100% | 88-96% |
| Cortical cysts | 47-78% | 10-26% |
| Tubular casts | 24-54% | 30-60% |

Only a single mouse had evidence of a neoplasm in the kidney (a mid-dose group female) and this was "metastatic sarcoma" and not a specific kidney tumor.

CONCLUSION (kidney): The kidney weight changes are not supported by pathological findings. There was no evidence of dose related necrosis.

c. Heart - Heart weight was decreased for the high-dose males and females.

The pathology of the heart indicated mononuclear inflammation and fibrosis and several other conditions of low incidence without evidence of a dose-related effect. There were no neoplasms reported in the hearts.

CONCLUSION (heart): The heart weight changes were not accompanied by pathological changes.

d. Brain - Brain weight was either decreased (female absolute weight for the high-dose group) or increased (male and female high-dose group and female low-dose group for relative body weight). Only a few incidences (i.e., up to 4 per dose group for mineralization) were reported in the brain without regard to a dose response. No neoplasms were reported in the brain.

CONCLUSION (brain): The brain weight changes were not supported by pathological changes.

e. Pituitary - The pituitary was demonstrated to have increased neoplasms in the rat TPTH oncogenicity study (RCC No. 646980, April 18, 1989) and to have dose-related increased incidence of non-neoplastic lesions. A study in rats with the structural analog tributyltin (WHO study, February 1988) also indicated that the pituitary was a target organ for a neoplastic effect of an organotin compound. The following table illustrates the histopathological findings in the mouse study in the RCC study currently under review.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| Lesion | No. | 49/50[1] | 8/27 | 15/26 | 50/48 |
| Adenomas | M | 0 | 0 | 0 | 0 |
| | F | 2 | 1 | 5 | 0 |
| Hyperplasia | M | 0 | 0 | 0 | 0 |
| | F | 11 | 6 | 7 | 2 |
| Cysts[2] | M | 6 | 0 | 0 | 4 |
| | F | 3 | 1 | 0 | 3 |

[1]/number of males/number of females examined.
[2]/In the rat there was a dose-related increase in the incidence of "cystoid change." No such lesion type is described for the mice. Thus cysts are considered the most closely related lesion and are included here.

There is no evidence that the pituitary of mice is a target organ for TPTH for either a neoplastic or non-neoplastic effect. Among the females, there were 5/26 mice with adenomas (19.23%) for the mid-

dose group but only 2/50 or 4% for the controls. This might suggest an effect but there were zero mice with adenomas in the high-dose group leading TB-I to the conclusion that the pituitary is not a target organ for on oncogenic effect of TPTH.

f. Testes - The testes was regarded as a target organ for both non-neoplastic (Leydig cell hyperplasia and tubular atrophy) and neoplastic (Leydig cell tumors) in the rat (RCC Study No. 046980, April 18, 1989). The following table illustrates the findings in the testes in the mouse study.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| Lesion | No. | 50 | 13 | 18 | 50 |
| Tubular atrophy | | 17 | 8 | 7 | 25NS |
| Mineralization | | 13 | 4 | 4 | 8 |
| Interstitial Hyperplasia | | 4 | 1 | 0 | 0 |
| Interstitial Cell Tumor | | 3 | 0 | 0 | 0 |

There is no evidence that the testes was a target organ for either a neoplastic or non-neoplastic effect of TPTH in the mouse.

g. Thymus - The thymus is a part of the immune system and TPTH is being investigated as a possible immunotoxic agent. Immunoglobulins were decreased in this study.

In this mouse study the following lesion types (plus others of frequency of up to 3 per group) were reported.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| Lesion | No. | 42/45[1] | 42/49 | 45/46 | 38/41 |
| Thymic Involution | M | 27 | 19 | 25 | 34** |
| | F | 12 | 13 | 14 | 30*** |
| Medullary Cysts | M | 29 | 20* | 20* | 16* |
| | F | 8 | 6 | 3 | 16* |
| Thymic | M | 0 | 16 | 12 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Hyperplasia | F | 15 | 10 | 7 | 6 |

[1] number of males/number of females examined.
* P < 0.05, ** P < 0.01 and *** P < 0.001. Fisher's Exact P. HED Computer.

Of these lesions there is an apparent increase in "thymic involution" for both males (64% for the control vs. 89.5%) and females (26.7% for the controls vs. 73.2%) high-dose groups. The low- and mid-dose groups appear to be in the range of the control. Medullary cysts were decreased for males and increased for females. Hyperplasia also was increased for the mid and high dose male groups but decreased for females.

CONCLUSION (thymus). The lesion types involved are considered by TB-I to have a wide distribution and although some changes are apparent, there is insufficient basis to conclude they are related to TPTH toxicity. The lesion "thymic involution" is a vague morphological description probably associated with the natural regression of this organ and is not considered to be related to the immunoglobulin level changes.

h. Spleen - The spleen is involved in the immune system and TPTH is being investigated as an immunotoxin.

In this study, many of the mice had conditions described as "increased granulopoiesis" (males 22 to 44%, females 28 to 42%), increased erythropoiesis (males 10 to 50%, females 14 to 78%) and lymphoid hyperplasia (males 4 to 22%, females 8 to 30%). None of these showed a dose response increase. The lesion described as increased erythcpoieses showed a marked decrease (78%) for the control vs. 42 percent for the high-dose group).

In conclusion, the changes in immunoglobulin levels are not supported by pathological changes in the spleen.

i. Adrenals - The adrenals were indicated as a target organ for tributyltin, an organotin analog, in a rat study (WHO, 1988).

There was a single incidence of adrenal carcinoma in this mouse study (low-dose group female). There were a variety of non-neoplastic lesions reported in the adrenals but none of these showed evidence of a definite dose response. The lesion described as "lipogenic pigment" was present in the high dose

(74%) more than in the controls (53%) for the males, but for females both the controls and high-dose group had 86 percent. TB-I does not consider this as an effect in the males.

j. Uterus - The uterus was reported to have a dose-related increase in "endometrial hyperplasia" in an earlier mouse oncogenicity study, now since determined to be INVALID (Cannon Laboratories, No. 6E-725, August 28, 1978). The following table illustrates the major pathological findings in the uterus in the current mouse study.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| Lesion | No. | 50 | 36 | 36 | 49 |
| Dilated Lumen | | 2 | 16*** | 12*** | 15*** |
| Cystic Change | | 39 | 24 | 20* | 15*** |

As indicated above, the three groups dosed with TPTH have higher incidences of "dilated lumen" but there is no dose response over the broad range of 5 to 80 ppm. TB-I does not consider the apparent increase in incidence of "dilated lumen", a common condition in the uterus, to be a response to TPTH treatment. The lesion described as "cystic change" shows an apparent dose related decrease that is not considered to be of toxicological significance.

CONCLUSION (uterus): The uterus is not regarded as a target organ in the mouse for TPTH based on the data presented in this study.

k. Skin - Necropsy indicated that lesions in the female high-dose group had more incidences of the skin which were described as sores and eschars. The following table illustrates the pathological findings for some lesions possibly showing increased incidence with the high dose level of TPTH.

| | | Control | 5 ppm | 20 ppm | 80 ppm |
|---|---|---|---|---|---|
| Lesion | No. | 50/50 | 13/27 | 29/29 | 50/50 |
| Dermatitis | M | 1 | 2 | 9** | 7* |
| | F | 7 | 3 | 10* | 17* |
| Follicular Keratosis | M | 1 | 0 | 2 | 0 |
| | F | 1 | 0 | 3 | 4 |
| Acanthosis | M | 4 | 2 | 1 | 2 |
| | F | 1 | 2 | 6** | 11** |
| Hyperkeratosis | M | 4 | 2 | 1 | 2 |
| | F | 1 | 2 | 6** | 11** |

* P < 0.05, ** P < 0.01, *** P < 0.001. Fisher's Exact P. HED Computer.

The above table implies a NOEL of 5 ppm. At 20 ppm there is increased incidence of dermatitis (males and females), follicular keratitis (females), acanthosis and hyperkeratosis (females).

10. Maximum Tolerated Dose (MTD) Considerations - Based on the increased incidence of deaths in females and body weight decreases in bcth sexes, the MTD was attained. It is possible that the liver tumors in females in the high-dose group developed at a dose level in excess of the MTD. This will be reconsidered in the Peer Review for Oncogenicity of TPTH.

CONCLUSION. This study is classified CORE GUIDELINE. This study demonstrates evidence of oncogenicity based on increased incidence of liver adenomas (males and females) and carcinomas (females).

At 5 ppm and above there were decreases in immumoglobulins.

At 20 ppm and above: increases in body weight (females, first months of study), kidney weight decreases (without pathological changes). Skin lesions.

At 80 ppm: deaths and changes in appearance (females), decreased weight gain and liver weight increases.

HISTORICAL CONTROL TUMOR INCIDENCE
NMRI MOUSE

007501

## ORGAN: LIVER

### HEPATOCELLULAR ADENOMA

| PROJECT | STUDY TYPE | REPORT | PATH. | No. OF ORGANS EXAMINED | | No. OF ORGANS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 027810 | 79 WEEK FEEDING | 1987 | HHW | 50 | 49 | - | - | 0 | 0 |
| 000426 | 80 WEEK FEEDING | 1983 | HJC | 50 | 50 | - | - | 0 | 0 |
| 047002 | 80 WEEK FEEDING | 1988 | JMA | 49 | 49 | 6 | - | 12 | 0 |
| 006388 | 96 WEEK FEEDING | 1985 | HJC | 50 | 50 | 4 | 1 | 8 | 2 |
| 000911 | 104 WEEK FEEDING | 1984 | PAG | 50 | 50 | 3 | 1 | 6 | 2 |
| 008796 | 104 WEEK FEEDING | 1984 | HHW | 50 | 50 | 3 | - | 6 | 0 |
| 004263 | 104 WEEK FEEDING | 1985 | PAG | 50 | 50 | 6 | 1 | 12 | 2 |
| 005275 | 104 WEEK FEEDING | 1985 | JAW | 50 | 50 | 2 | - | 4 | 0 |
| 005310 | 104 WEEK FEEDING | 1985 | JMA | 50 | 49 | 7 | 2 | 14 | 4 |
| 008853 | 104 WEEK FEEDING | 1985 | BSC | 50 | 50 | 5 | - | 10 | 0 |
| 014398 | 104 WEEK FEEDING | 1986 | JAW | 50 | 50 | 6 | 1 | 12 | 2 |
| 018527 | 104 WEEK FEEDING | 1986 | BSC | 50 | 50 | 8 | - | 16 | 0 |

### HEPATOCELLULAR CARCINOMA

| PROJECT | STUDY TYPE | REPORT | PATH. | No. OF ORGANS EXAMINED | | No. OF ORGANS WITH TUMORS | | INCIDENCE IN % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F |
| 027810 | 79 WEEK FEEDING | 1987 | HHW | 50 | 49 | 3 | - | 6 | 0 |
| 000426 | 80 WEEK FEEDING | 1983 | HJC | 50 | 50 | 1 | - | 2 | 0 |
| 047002 | 80 WEEK FEEDING | 1988 | JMA | 49 | 49 | 2 | - | 4 | 0 |
| 006388 | 96 WEEK FEEDING | 1985 | HJC | 50 | 50 | 1 | - | 2 | 0 |
| 000911 | 104 WEEK FEEDING | 1984 | PAG | 50 | 50 | 2 | - | 4 | 0 |
| 008796 | 104 WEEK FEEDING | 1984 | HHW | 50 | 50 | - | - | 0 | 0 |
| 004263 | 104 WEEK FEEDING | 1985 | PAG | 50 | 50 | 2 | 1 | 4 | 2 |
| 005275 | 104 WEEK FEEDING | 1985 | JAW | 50 | 50 | - | - | 0 | 0 |
| 005310 | 104 WEEK FEEDING | 1985 | JMA | 50 | 49 | - | - | 0 | 0 |
| 008853 | 104 WEEK FEEDING | 1985 | BSC | 50 | 50 | 1 | - | 2 | 0 |
| 014398 | 104 WEEK FEEDING | 1986 | JAW | 50 | 50 | 4 | - | 8 | 0 |
| 018527 | 104 WEEK FEEDING | 1986 | BSC | 50 | 50 | 2 | - | 4 | 0 |