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

005299

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUL 15 1986

MEMORANDUM

SUBJECT: EPA Registration No. 8340-17: Review of Rat and Mouse Subchronic Feeding Studies with Triphenyltin Hydroxide

TOX Chem No. 896E
TOX Project No. 1364
Record No. 169141

FROM: John Doherty 7/9/86
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Henry M. Jacoby, PM 21
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THRU: Edwin R. Budd, Section Head 7/11/86
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

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Hazard Evaluation Division (TS-769C)

Background:

The American Hoechst Corporation has submitted 90-day rat and mouse feeding studies which were conducted to assess dose levels for chronic feeding and/or oncogenicity studies with

triphenyltin hydroxide (TPTH). These studies were reviewed by Toxicology Branch (TB) as follows:

Toxicology Branch Comments:

1. Neither the rat nor the mouse study provided convincing evidence that the data demonstrate NOEL's for these species. In both species the immune system shows evidence of effects of exposure to TPTH at the lowest dose levels tested.

   The rat study shows indications of decreased IgG levels at all dose levels -41, -44 and -56 percent for the low-, mid-, and high-dose groups respectively for females. The male groups are also lower but statistical significance was not attained.

   In the mouse study, there were trends for decreases of all three ($I_gA$, $I_gM$ and $I_gG$) immunoglobulins assessed (see R. Levy report dated June 9, 1986 attached) such that TB could not assign a NOEL for potential effects on the immune system for this species.

   Special immunotoxicity studies will have to be conducted to assess for the immunotoxic potential of TPTH.

2. TB has questions regarding the quantitation and handling of the data related to the immunoglobulins in both the mouse and rat studies as presented by the study reports. These are as follows:

   a. The individual readings for the immunoglobulin data are in apparent quantal units with the same numbers appearing again and again. Because the immunoglobulins are proteins and their serum concentration depends on several factors, one would expect a normal distribution.

      The registrant is requested to explain why the data were distributed with the same values recurring as presented for the immunoglobulin determinations.

   b. Many values were presented as < (a specified value) meaning that no immunoglobulin could be detected for that sample. Such data were not included when the statistics were done on the data set. TB finds this a questionable practice. For example, the IgA data for the 100 ppm female group which had ten animals, had only eight values presented. Of these eight, seven were < 44.4 and one was 150.0. The single value of 150 was entered in the summary

thus misleading the results of the analysis. The registrant is requested to provide justification for not including values designated with < in the statistical processing of the data.

3. According to the letter dated February 26, 1986 from Dr. Berthold Volger, the oncogenicity studies with both rats and mice with TPTH are currently underway and apparently using the dose levels of 0, 4, 20, and 100 ppm. Since TB has not concurred with the assignment of the NOEL's set for these studies by the study reports, there is the possibility that the studies underway will not show NOELs. The NOELs which TB has set are tentative conclusions which will be reevaluated pending receipt and review of the chronic feeding and/or oncogenicity studies. The final reports of these long term studies must fully address the possibility that TPTH affects the immunoglobulin levels and any other endpoints of the immune system. The registrant, if it wishes, may consider including extra rats or mice at this time in the studies (both control and test dose) to include a dose level that is lower than the current 4 ppm low dose group.

4. In addition to the potential immunotoxicity problems indicated above, the final reports of the rat and mouse chronic feeding and/or oncogenicity studies must address the following items in particular.

   i. significance of the increase in aspartate aminotransferase in the blood in the rat study.

   ii. mean corpuscular volumes and mean corpuscular hemoglobin levels should be carefully assessed for possible decreases in the rat study.

   iii. decreases in ovary and adrenal weight and increases in liver weight in the mouse study.

5. On the basis of the data presented it appears that the rat study can predict a Maximum Tolerated Dose (MTD) to be used for the chronic feeding/oncogenicity study because the high dose test group results in body weight decreases of 7% in males and 4% in females. It should be noted that both of these weight decreases are not at the criteria of a 10% decrease. The mouse study indicates that the dose levels of 100 ppm may not reach the MTD because there were no definite toxic responses or decreases in body weight.

Studies Reviewed

| | | |
|---|---|---|
| 90-day feeding - rats | Tentative conclusion: NOEL < 4 ppm (0.33 mg/kg/day, average for both sexes) decreased incidences of IgG antibodies. Conclusion subject to reassessment after receipt and review of rat chronic feeding study.<br><br>NOEL for systemic effects not including effects on the immune system = 1.64 mg/kg/day (20 ppm).<br><br>LEL = 7.63 mg/kg/day (100 ppm), decreases in body weight gain and food consumption in males and females and possible increase in ASAT. | GUIDELINES |
| 90-day feeding - mice | NOEL for potential immunotoxic effects not established but < 0.75 mg/kg/day (4 ppm), special testing for immunotoxicity will be required.<br><br>NOEL* for systemic effects, not including immunotoxic effects, = 0.75 mg/kg/day (4 ppm).<br>LEL* = 3.78 mg/kg/day (20 ppm) decreased adrenal weight in females.<br><br>LEL* = 19.46 mg/kg/day (100 ppm) decreased ovary weight, increased liver weight in both males and females. | MINIMUM |

*Note: THese organ weight changes (adrenal and ovary) occurred without accompanying pathological changes. Thus the conclusions regarding the NOEL and LEL for systemic effects are tentative and subject to confirmation in the mouse oncogenicity study.

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Reviewed by: J.D. Doherty
Section 2, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd
Section 2, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: 13-Week Oral Feeding-Rats TOX. CHEM. NO.: 896E

Accession Number: 261754 MRID NO.: ~~N/A~~ 157771

Test Material: Triphenyltin Hydroxide

Synonyms: TPTH

Study Number(s): 046978 ~~Data~~

Sponsor: American Hoechst

Testing Facility: Research and Consulting Company AG (Itingen, Switzerland)

Title of Report: 13-Week Oral Toxicity (Feeding) Study with TPTH Technical

Author(s): P. Suter, K. Horst, W. Vogel, H. Luetkemeier, G. Pappritz, Ch. Terier, K. Sachsse

Report Issued: January 15, 1986

Conclusions: (See Discussion)

Classification: Core-GUIDELINES

A. Materials:

1. Test compound: Triphenyltin Hydroxide, Description: Powder, Batch #02782, Purity 97.2%, contaminants: not identified.

2. Test animals: Species: rats; Strain: Wistar; Age: 4-5 weeks; Weight: 92-117g males, 71-95g females. Source: KFM Kleintierfarm Madoerin AG. There was a seven day aclimatization period and the rats were five per cage.

B. Study Design:

1. Animal assignment - Animals were assigned by computer generated random algorithm to the following test groups:

| Test Group | Dose in diet (ppm) | Main Study 3 months male | Main Study 3 months female | Recovery group 1 month* male | Recovery group 1 month* female |
|---|---|---|---|---|---|
| 1. Cont. | 0 | 10 | 10 | 5 | 5 |
| 2. Low (LDT) | 4 | 10 | 10 | 5 | 5 |
| 3. Mid (MDT) | 20 | 10 | 10 | 5 | 5 |
| 4. High (HDT) | 100 | 10 | 10 | 5 | 5 |

*Dosed with the test material for 13 weeks followed by a 4 week recovery period.

2. Diet preparation - Diet was prepared twice monthly by mixing and pelleting the food and stored at room temperature. Samples of treated food were analyzed for homogeneity and concentration monthly.

Results - Diets shown to be about 25% below the nominal concentration as follows:

4 ppm - 83.6 $\pm$ 24.6%
20 ppm - 75.9 $\pm$ 4.2%
100 ppm - 77.5 $\pm$ 7.8%

3. Animals received food (pelleted rat diet) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Univariate one-way analysis, Dunnett-test, Steel test and Fisher's exact test.

5. Quality assurance was monitored by periodic inspections (5) by K. Schneider.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Weekly palpations were made.

No treatment related deaths or signs of overt or obvious toxicity were noted. Two females in the mid dose group died after blood sampling.

2. Body weight - The rats were weighed weekly for 13 weeks.

Results - Only the high dose test group showed decreases. For example, males were about 7 percent lower and females were about 4 percent lower. After recovery, the males were about 1-2 percent lower and the females were still about 4 percent lower (of doubtful significance).

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and nominal concentrations of the test material in the diet.

Results - Food consumption - High dose group was slightly lower than controls.
Food efficiency - Not obviously affected.
Compound intake - (in mg/kg/day)

| | Males | Females |
|---|---|---|
| Control | 0 | 0 |
| 4 ppm | 0.3 | 0.35 |
| 20 ppm | 1.56 | 1.72 |
| 100 ppm | 7.41 | 7.85 |

4a. Ophthalmological examinations were performed at pretest, and at weeks 4 and 13 on all animals.

Results - No treatment related differences were noted.

b. Hearing tests were performed at pretest, and at weeks 4 and 13 on five rats per group. The method consisted of using a tone generator (volume 80dB, frequency of 10 KHZ of 30 seconds duration repeated five times with 2-second pauses. Observation of a Preyer's Reflex was considered to be positive reaction.

No adverse effects on the hearing of the rats were detected.

5. Blood was collected at 13 weeks and after 4 weeks for recovery for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

This is an important aspect of TPTH toxicity because TPTH is potentially immunotoxic and previous studies have shown that TPTH decreases the white blood cell counts.

a. Hematology -

| X | | X | |
|---|---|---|---|
| X | Hematocrit (HCT) | | Total plasma protein (TP) |
| X | Hemoglobin (HGB) | X | Leukocyte differential count |
| X | Leukocyte count (WBC) | X | Mean corpuscular HGB (MCH) |
| X | Erythrocyte count (RCB) | X | Mean corpuscular HGB conc. (MC |
| X | Platelet count | X | Mean corpuscular volume (MCV) |
| X | Reticulocyte count | X | Coagulation time |
| X | Heinz body | X | Normoblasts |
| X | Red Cell Morphology | | |

Results - The mean corpuscular volume (both mid and high dose group males, -4% and -5%, and high dose group females -4%) was decreased. The mean corpuscular hemoglobin for both males (-4%) and females (-4%) for the high dose groups was decreased after 13 weeks but was equivalent to controls after the recovery period. The magnitude of the decrease in RBC parameters is too small to be considered a definite toxicity response. Since both sexes were decreased in the high dose group this parameter should be carefully and fully examined in the 2 year rat study.

The changes in the white blood cell counts reported are shown in the following Table

White Blood Cells (in G/l)

| | Males | | Females | |
|---|---|---|---|---|
| | 13 weeks | 17 weeks | 13 weeks | 17 weeks |
| Control | 7.0 | 6.3 | 5.0 | 4.7 |
| Low Dose | 7.8 | 6.8 | 4.5 | 4.7 |
| Mid Dose | 6.4t | 6.1 | 3.9* | 4.3t |
| High Dose | 6.6t | 6.9 | 3.8* | 4.0t |

*statistically significant at the 5% level.
t not significant but supports a trend.

There is some evidence that the white blood cell counts in the females decreases as a response to TPTH. The previously observed marked decreases (refer to the Registration Standard for TPTH) in white blood cell counts in male rats were not noted in this study.

The potential effects of TPTH on white blood cell counts is not resolved by this study.

b. Clinical Chemistry (items marked X were determined).

| | Electrolytes: | | Other: |
|---|---|---|---|
| X | Calcium | | Albumin |
| X | Chloride | X | Blood creatinine |
| | Magnesium | X | Blood urea nitrogen |
| X | Phosphorous | X | Cholesterol |
| X | Potassium | | Globulins |
| X | Sodium | X | Glucose |
| | Enzymes | X | Total Bilibrubin (total and direct) |
| X | Alkaline phosphatase | X | Total Protein (electrophorsis) |
| | Cholinesterase | | Triglycerides |
| X | Creatinine phosphokinase | X | $I_gG$ immunoglobulins |
| X | Lactic acid dehydrogenase | | |
| X | Serum alanine aminotransferase (also SGPT) | | |
| X | Serum aspartate aminotransferase (also SGOT) | | |
| X | Gamma glutamyl transferase | | |
| X | Ornithine carbamyl transferease | | |

Results - The study report showed that there were slightly increased levels of aspartate aminotransferase (ASAT, 24% in males and 13% in females) and alkaline phosphatase (ALP, 17% in males and 29% in females) in the high dose groups at 13 weeks. These enzyme values were also slightly elevated after 17 weeks.

The toxicological significance of these increases are considered indefinite because of the small incremental increase. The elevations in these enzyme activities may, however, indicate tissue injury. It is however, disturbing that both sexes in the high dose test groups showed increases in ASAT.

Other clinical chemistry values that showed deviations form the control readings were calcium (decreases of 3% in the mid and high dose males and females). The protein electrophoresis data also showed that there were both increases and decreases in the readings of the protein types relative to the control values. No toxicological significance is assigned to this finding.

The females (but not the males) exhibited a possible effect on the immune system as reflected by the decreases in IgG noted only in the "recovery group" as shown in the following table.



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| | Males | | Females | |
|---|---|---|---|---|
| | 13 weeks | Recovery | 13 weeks | Recovery |
| | IgG in mg/100 mL | | | |
| Control | | | | |
| 0 ppm | 451 | 545 | 496 | 370 |
| 4 ppm | 568 | 507 | 446 | 219* |
| 20 ppm | 345 | 349 | 467 | 207* |
| 100 ppm | 367 | 331 | 445 | 163* |

* statistically significant by the study report's calculations ($p<0.05$). Data are the average determined for each group of upt to 5 rats.

|—| supports a trend toward a decrease as interpreted by TB reviewer.

The study report asserts that the significance of the decrease in IgG in the recovered animals is "unclear" but that theoretically this might suggest the involvement of a delayed immunosuppressive response or decreased IgG synthesis.

It should be noted here that the mouse 90-day study (see following review) also shows a decrease in IgG and other immunoglobulins.

6. Urinalysis - Urine was collected from fasted animals at 13 months. The CHECKED (X) parameters were examined.

| | | | |
|---|---|---|---|
| X | Appearance | X | Glucose |
| X | Volume | X | Ketones |
| X | Specific gravity | X | Bilirubin |
| X | PH | X | Blood |
| X | Sediment (Microscopic) | X | Nitrate |
| X | Protein | X | Urobilinogen |

Results - Only "slight" variations in urine volume (increases), specific gravity (decreases), and pH value (increases) in group 4 (high dose groups) were observed. These differences are of indefinite significance.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues

were collected for histological examination. The (XX) organs in addition were weighed.

| X | Digestive system |
|---|---|
| X | Tongue |
| X | Salivary glands |
| X | Esophagus |
| X | Stomach |
| X | Duodenum |
| X | Jejunum |
| X | Ileum |
| X | Cecum |
| X | Colon |
| X | Rectum |
| XX | Liver |
| | Gall bladder |
| X | Pancreas |
| | Respiratory |
| X | Trachea |
| X | Lung |

| X | Cardiovasc./Hemat. |
|---|---|
| X | Aorta |
| XX | Heart |
| X | Bone marrow |
| X | Lymph nodes |
| XX | Spleen |
| X | Thymus |
| | Urogenital |
| XX | Kidneys |
| X | Urinary bladder |
| XX | Testes |
| X | Epididymides |
| X | Prostate |
| X | Seminal vesicle |
| XX | Ovaries |
| X | Uterus |

| X | Neurologic |
|---|---|
| XX | Brain |
| | Periph. nerve (sciatic) |
| X | Spinal cord (3 level) |
| XX | Pituitary |
| X | Eyes (optic n.) |
| | Glandular |
| XX | Adrenals |
| | Lacrimal gland |
| X | Mammary gland |
| | Parathyroids |
| XX | Thyroids |
| | Other |
| X | Bone (sternum) |
| X | Skeletal muscle |
| X | Skin |
| X | All gross lesions and masses |
| X | Harderian glands |

Results -

a. Organ weight - Organ weight and organ weight ratios relative to both the body weight and brain weight were determined.

Of particular concern for TPTH are the spleen and thymus weights because these organs have been shown to be affected in other studies with TPTH. The thymus was not weighed for this study.

Spleen weights were not affected in this study (no group reached the $p < 0.05$ percent level of statistical significance (Dunnett-test) although there were some indications of decreased weight as well as increased weight for some groups. It must be remembered that since the spleen is a vascular organ, weight determinations may vary because of the blood content.

None of the other organs showed a definite test chemical effect. The pituitary for both the male and female high dose groups was lower slightly in weight (10% males and 14% females). The adrenal weights of the females were also elevated in the high dose group but this group had a large standard deviation and was not statistically significant.

b. Gross pathology

The report states that "no treatment related macroscopic findings were observed."

TB notes however, that there is no summary table showing the types and frequency of macroscopic lesions that were present in the test animals. The individual animal pathology sheets clearly indicate what types of macroscopic pathology was present for each rat.

c. Microscopic pathology

1. Non-neoplastic

The report states that "no treatment-related microscopic findings were observed."

The summary table shows that only the control and high dose test groups (10 rats of each sex) were examined microscopically) except as followup to gross lesions. No evidence of TPTH induced increases in lesion types was evident.

2. Neoplastic

No tumors were noted, a finding consistent with the 90 day duration of the study.

D. Discussion:

The testing laboratory asserts a NOEL of 20 ppm and a LEL of 100 ppm (or 1.56 mg/kg/day for males and 1.72 mg/kg/day for females). The only test chemical effects recognized by the study report are decreases in body weight gain at 100 ppm.

TB does not concur at this time with this assignment for the NOEL. TB has set a temporary NOEL of < 4 ppm for this study based on the decreases in IgG concentration in the blood. Because of the known potential of organotin chemicals to affect the various parameters of the immune system, TB cannot ignore the decreases noted in this parameter. The NOEL is tentatively assigned pending receipt and review of the chronic feeding study with TPTH which may either confirm this finding or otherwise demonstrate that the decrease as noted was incidental.

The rat chronic feeding/oncogenicity study should carefully assess the immunoglobulin levels as well as other as-

pects of the immune system and carefully assess for possible effects of TPTH on ASAT activity and for effect on the red blood cells.

Based on the changes noted in body weight gain, it is apparent that the highest dose level tested here (100 ppm groups) is in the range of the Maximum Tolerated Dose (MTD).

One Liner:

NOEL < 0.33 mg/kg/day (4 ppm) average for both sexes. Decreased concentration of IgG anbtibodies. Conclusion subject to reassessment after receipt and review of the rat chronic feeding study.

NOEL for systemic effects not including effects on the immune system = 1.64 mg/kg/day (20 ppm).

LEL = 7.63 mg/kg/day (100 ppm) decreases in food consumption and body weight gain in males and females and possible increase in ASAT.

GUIDELINES

005299

Reviewed by: J.D. Doherty
Section 2, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd
Section 2, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Subchronic Feeding-13 Week-Mouse TOX. CHEM. NO.: 896E

Accession Number: 261753 MRID NO.: ~~N/A~~ 157952

Test Material: Triphenyltin Hydroxide

Synonyms: TPTH

Study Number(s): 046991

Sponsor: American Hoechst

Testing Facility: Research and Consulting Company (RCC) Itingen, Switzerland

Title of Report: 13-Week Oral Toxicity (Feeding) study with TPTH Technical

Author(s): P. Suter, K. Horst, W. Vogel, H. Luetkemeier, G. Pappritz, Ch. Terier, K. Sachsse

Report Issued: January 20, 1986

Conclusions: See Discussion and One Liner

Classification: Core-MINIMUM [There were deficiencies in the number of investigational endpoints to qualify this study as Guidelines for a subchronic study.]

A. Materials:

1. Test compound: Triphenyltin Hydroxide, Description: Powder, Batch #02782, Purity 97.2%, contaminants: not provided.

2. Test animals: Species: mice, Strain: NMRI, Age: 5 weeks, Weight: 26-30 g males, 21-25 g females, Source: KFM Kleintierfarm Madoeim AG Switzerland. There was a seven day aclimatization period. The mice were housed individually.

B. Study Design:

1. Animal assignment - Animals were assigned by computer generated algorithm to the following test groups:

| Test Group | Dose in diet (ppm) | Main Study 3 months male | Main Study 3 months female | Interim Sac. months male | Interim Sac. months female |
|---|---|---|---|---|---|
| 1. Cont. | 0 | 10 | 10 | N/A | |
| 2. Low (LDT) | 4 | 10 | 10 | | |
| 3. Mid (MDT) | 20 | 10 | 10 | | |
| 4. High (HDT) | 100 | 10 | 10 | | |

2. Diet preparation - Diet was prepared by pelletizing and stored at room temperature. Samples of treated food were analyzed for homogeniety, stability and concentration,

3. Animals received food (pelleted mouse diet) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Univariate one-way analysis, Dunnett-test, Steel test, Fisher's exact test.

5. Quality assurance was assessed five times throughout the study by K. Schneider, Manager, Quality Assurance Unit.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. They were palpated weekly.

Results
Mortality (survival): No treatment related mortality resulted, however, one male (group 2) and one female in the control and in group 4 died after blood sampling.

Toxicity: No test article-related clinical signs or toxic symptoms were reported in any of the test groups.

2. Body weight - The mice were weighed weekly for 13 weeks.

Results - No significant body weight differences were reported.

3. Food consumption and compound intake Compound intake was calculated from the consumption and nominal concentrations of test material in the diets.

Results - Food consumption - No differences between controls and test animals

Compound intake - The following table shows the mean compound intake for both sexes for this study (in mg/kg/day).

| | Males | Females |
|---|---|---|
| Control | 0.00 | 0.00 |
| 4 ppm | 0.62 | 0.88 |
| 20 ppm | 3.44 | 4.12 |
| 100 ppm | 18.28 | 20.64 |

4a. Opthalmological examinations were not performed.

5. Blood was collected before treatment and at 13 weeks for hematology and clinical analysis from 10 animals/sex/group. The CHECKED (X) parameters were examined.

a. Hematology -

| X | |
|---|---|
| X | Hematocrit (HCT) |
| X | Hemoglobin (HGB) |
| X | Leukocyte count (WBC) |
| X | Erythrocyte count (RCB) |
| X | Platelet count |

| X | |
|---|---|
| | Total plasma protein (TP) |
| X | Leukocyte differential count |
| X | Mean corpustular HGB (MCH) |
| X | Mean corpustular HGB conc. (MC |
| X | Mean corpustular volume (MCV) |
| X | Reticulocyte Count |
| X | Nucleated erythrocytes |
| X | Heinz bodies |
| X | Red Cell Morphology |

The test report asserts that there were no changes in these parameters of toxicological importance.

TB has noted some deviations from the control group values especially in the high dose group. Because TPTH is being investigated in particular for possible effects on blood, these deviations are indicated as follows even though they are of a small magnitude.

i. RBCs decreased 7% in males (significant, 5% level) and 5 % in females (not significant).

ii. Hemoglobin was decreased in males (10%) and females (also 10%) and both were statistically significant (5%).

iii. Platelet levels were increased in both males (15%) and females (26%) and both were statistically significant (5%)*.

iv. Mean cell hemogloblin (-4%) and mean cell hemoglobin concentation (-6%) were decreased in females and both were statistically significant (5%).

v. The white blood cells in males have an unusual distribution with the low dose group being 38% (significant) lower than the control but the high dose group being only 20% (not significant) less than the control.

The oncogenicity study in mice should clarify the potential for TPTH to affect any of these parameters.

b. Clinical Chemistry (limited assessments made as follows)

| X | Electrolytes: |
|---|---|
| | Calcium |
| | Chloride |
| | Phosphorous |
| | Potassium |
| | Magnesium |
| | Sodium |
| | Enzymes |
| | Alkaline phosphatase |
| | Cholinesterase |
| | Creatinine phosphokinase |
| | Lactic acid dehydrogenase |
| | Serum alanine aminotransferase (also SGPT) |
| | Serum aspartate aminotransferase (also SGOT) |

| X | Other: |
|---|---|
| | Albumin |
| | Blood creatinine |
| | Cholesterol |
| | Globulins |
| | Blood urea nitrogen |
| | Glucose |
| | Total Bilibrubin |
| X | Total Protein |
| | Triglycerides |

| | |
|---|---|
| X | Protein electrophoresis |
| X | Immunoglobulins IgG, IgA, IgM |

Results - The study report asserts that there were no "changes of toxicological significance after 13 weeks of treatment." There were, however, some small changes in the following parameters (at the HDT):

- slightly increased total protein for female mice

*Dunnett-test significant at the 5% level.

- slightly increased albumin and alpha-globulin fractions for females,
- slightly decreased gamma-globulin fraction for females,
- slightly increased albumin to globulin ratio of the protein electrophorectic pattern for female mice.
- slightly decreased IgG level for male mice and a trend for a lower IgG level for female mice.
- moderately decreased IgA level for male and female mice.
- slightly decreased IgM level for female mice.

The report interpreted the decreases in the immunoglobulins G, A and M to be suggestive of an immunosuppresive response or decreased synthesis of the immunoglobulins.

TB has assessed the data on the quantitation of the immunoglobulins and has prepared the following table:

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Group | IgG | IgA | IgM | IgG | IgA | IgM |
| Control | 90.2±30.9 (32–125) | 143±95 (44–289) | 7.5±2.1 (3.6–9.5) | 70.9±30.5 (60–130) | 122.7±45 (75.9–193) | 24±8.5 (9.5–42.8) |
| Low | 70.7±3.15 (30–130) | 106±66 (44–193) | 12.2±3.2 (9.5–18.8) | 90.6±55.9 ((57–235) | 88.7±45.6 (44.4–193) | 18.3±7.7 (5.6–29.9) |
| Mid | 87±53.7 (40–220) | 103.2±49 (44–193) | 9.3±4.3 (5.6–18.8) | 93.5±35.8 (32–130) | 64.0±27.9 (44.4–111) | 17.03±8.5 (5.56–29.9) |
| High | 53±7.5 * (40–64) | 44.4±0 (44.4) | 5.95±1.24 (5.6–9.5) | 42.5±12.3) (30–60) | 57.6±37.3 (44.4–150) | 8.01±3.49 (5.6–13.9) |

*Dunnett-test significant at the 5% level.
|___| Data enclosed support a trend.

These data were assessed by Mr. R. Levy, statistician, TB and his report (dated June 9, 1986) is attached. The following is a quote from the summary of Mr. Levy's report.

"Statistically significant ($p < .01$), decreasing, dose related, trends are found in male and female mice for immunoglobulins (IgG, IgA and IgM) after 13 weeks of TPTH treatment. Thus, the immuoglobulin data cannot be considered incidental and of normal biological variation. Therefore, the significant decrease in the immunoglobulins G, A, M, in both sexes, strongly suggest an immunosuppressive response or decreased synthesis of the immunoglobulins."

This statistical analysis of the data together with the known property of organotin derivatives to affect the immune system leads TB to the conclusion that these study data do not appropriately assess the effects of TPTH in the immune system and additional studies especially designed to assess for effects of TPTH in the immune system will be required.

TB has questions regarding the quantitation and handling of the data related to the immunoglobulins as presented by the study report. These are as follows:

1. The individual readings for the immunoglobulin data are in apparent quantal units with the same numbers appearing again and again. Because the immunoglobulins are proteins and their serum concentration depends on several factors, one would expect a normal distribution.

   The registrant is requested to explain why the data were distributed with the same values recurring as presented for the immunoglobulin determinations.

2. Many values were presented as < (a specified value) meaning that no immunoglobulin could be detected for that sample. Such data were not included when the statistics were done on the data set. TB finds this a questionable practice. For example, the IgA data for the 100 ppm female group which had ten animals, had only eight values presented. Of these eight, seven were < 44.4 and one was 150.0. The single value of 150 was entered in the summary table, thus misleading the results of the analysis.

   The registrant is requested to provide a justification for not including values designated with < in the statistical processing of the data.

6. Urinalysis - No urinalysis was made.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

| X | Digestive system | X | Cardiovasc./Hemat | X | Neurologic |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta | XX | Brain |
| X | Salivary glands | XX | Heart | X | Periph. nerve |
| X | Esophagus | | Bone marrow | X | (sciatic) |
| X | Stomach | X | Lymph nodes | X | Spinal cord (3 |

| | | | | | |
|---|---|---|---|---|---|
| X | Duodenum | X | Spleen | | level) |
| X | Jejunum | X | Thymus | X | Pituitary |
| X | Ileum | | Urogenital | X | Eyes (optic n.) |
| X | Cecum | XX | Kidneys | | Glandular |
| X | Colon | X | Urinary bladder | XX | Adrenals |
| X | Rectum | XX | Testes | | Lacrimal gland |
| XX | Liver | X | Epididymides | X | Mammary gland |
| | Gall bladder | X | Prostate | | Parathyroids |
| X | Pancreas | X | Seminal vesicle | X | Thyroids |
| | Respiratory | XX | Ovaries | | Other |
| X | Trachea | X | Uterus | X | Bone (sternum) |
| X | Lung | | | X | Skeletal muscle |
| | | | | X | Skin |
| | | | | X | All gross lesions and masses |
| | | | | X | Harderian glands |

Results -

a. Organ Weight

Absolute and relative liver weight was increased by the test chemical in both sexes but only the high dose test group showed an effect that was statistically significant ($p<0.01$). For example, the males were 51 percent higher and the females were 69 percent higher than the controls. The liver for the mid dose group males was 6 percent higher but this difference was not statistically significant.

Among the females, the adrenal weight at both 20 (absolute weight $p<0.05$) and 100 ppm (absolute and relative weight $p<0.01$) were depressed (-32% and -50%) and the ovary weight was depressed for the high dose group (-68%).

No data were presented on spleen weights; an important omission in terms of using this study to assess for immunotoxic effects.

b. Gross pathology

The report states that "no treatment-related macroscopic findings were observed", although a "few spontaneous gross lesions were encountered in both control and treated mice". No summary table was presented. The gross lesions that did occur are indicated on the individual animal pathology sheets.

c. Microscopic pathology

1. Non-neoplastic

Only the mice in the control and high dose test groups were assessed for microscopic pathology as well as those mice which showed obvious gross lesions in the low and mid dose test groups. The report states that "no-treatment related microscopic findings were observed."

The summary table (p. 137 of the report) indicates that although a variety of spontaneous lesions were present, their frequency in the control groups was equivalent to the frequency in the high dose test groups.

With the possible exception of mice with glycogen storage (four incidences in the controls vs. six incidences in the high dose group) there was no pathological state which accounted for the increase in liver weight. Similarly, the ovaries (one incidence of a cyst in the control group) and adrenals (no lesions reported) did not have pathological changes which accompanied their weight differences.

2. Neoplastic

No neoplasia reported, an expected finding for mice of this age.

D. Discussion:

The testing laboratory report asserts that the NOEL for this study is 20 ppm and the LEL is 100 ppm. At 100 ppm the report recognizes that some effects on parameters of the immune system were decreased (for example, IgG, IgA and IgM) and that liver weights for both sexes were elevated.

TB does not concur with the study report with respect to effects on the immune system. There are trends at all dose levels for each of the three parameters of the immune system investigated (see R. Levy report attached). Overall, the conclusion with respect to effects on the immune system is that the study does not appropriately assess the potential for TPTH to affect this physiological system. Thus, no NOEL for potential effects of TPTH on the immune system can be assigned based on these data. Special testing will be required.

TB also notes that in addition to the effects of TPTH recognized by the study report, adrenal weights in females in the mid and high dose groups and ovary weights in the high dose group were decreased, although, these organs did not have associated pathology.

It should be indicated here that the nominal dose levels used in this study were greater than the actual dose levels based on dietary analysis. Actual dose levels were about 80% for the mid and high dose level groups. The low dose group was about 94% of the nominal dose level.

The data from this study do not clearly establish a Maximum Tolerated Dose (MTD). There was no significant body weight change at the highest test dose level. The changes in the organ weights (adrenal and ovary) occurred without pathological changes meaning that the changes in weight may not have been directly related to dietary TPTH. There will be some possibility that the mouse study will not be conducted at the MTD if the highest dose level tested is 100 ppm.

<u>One Liner</u>

NOEL - not established for potential immunotoxic effects, special testing will be required. LDT = 0.75 mg/kg/day. MINIMUM

NOEL*- systemic effects (not including potential immunotoxic effects) = 0.75 mg/kg/day (average for both sexes)

LEL*= 3.78 mg/kg/day (20 ppm), decrease adrenal weight in females,

LEL*= 19.46 mg/kg/day (100 ppm), decreased ovary weight and increased liver weights in males and females.

*Tentative conclusion for systemic effects. The organ weight changes occurred without associated pathological changes.