8-6-91

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

AUG 6 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Triphenyltin hydroxide: Submission of documents for consideration by the Scientific Advisory Panel.

TOX CHEM No.: 896E
Shaunessey No.: 083601

FROM: John Doherty, Ph.D. 8/6/91
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: R, Bruce Jaeger
Secretary, Science Advisory Panel
Health Effects Division (H7509C)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

The following items concerning the carcinogenicity of triphenyltin hydroxide (TPTH) are attached for review by the Scientific Advisory Panel.

1. Peer Review Report dated May 24, 1990 prepared by Roy D. Sjoblad.

2. Peer Review Package dated November 5, 1989 prepared by John D. Doherty.

3. DER for the rat chronic feeding/carcinogenicity study conducted at the Research Consulting Company (RCC) and dated April 18, 1989.

4. DER for the mouse carcinogenicity study conducted at Research Consulting Company and dated April 24, 1989.

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D C 20460

MEMORANDUM May 24, 1990

SUBJECT: Peer Review of Triphenyltin hydroxide (TPTH)

FROM: Roy D. Sjoblad, Ph.D.
Science Analysis and Coordination Branch
Health Effects Division (H-7509C)

TO: Jack Housenger
Special Review and Reregistration Division (H7508C)

The Health Effects Division Peer Review Committee met on 11/29/89 to discuss and evaluate the weight-of-evidence on TPTH with particular reference to its carcinogenicity potential. The committee classified TPTH as a Group B2-Probable Human Carcinogen. Quantification of carcinogenicity risk is recommended at this time.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated).

Penny Fenner-Crisp	Penelope A. Fenner-Crisp
Reto Engler	
Karl Baetcke	
Marcia van Gemert	Marcia van Gemert
John A. Quest	John A. Quest
Esther Rinde	Esther Rinde
Kerry Dearfield	Kerry Dearfield
Marion Copley	Marion Copley
Robert Beliles	Robert Beliles
George Ghali	G. Ghali
Julie Du	
Yin-Tak Woo	Yin Tak Woo
	Ann L. Brun

2. Reviewers: (Non-committee members responsible for data presentation; Signatures indicate technical accuracy of panel report).

Roger Gardner — Roger Gardner 4/24/90

John Doherty — 4/24/90

Bernice Fisher — Bernice Fisher 4/25/90

Hugh Pettigrew — Hugh Pettigrew 4/25/90

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee).

Richard Hill —

William Sette — William Sette

Richard Levy —

4. Other Attendees (observers): Linda Kutney (HED)

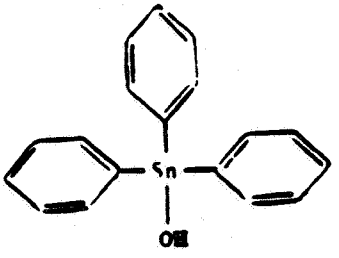
B. Material Reviewed:

The material available for review consisted of DERs for a rat oncogenicity study, and a mouse oncogenicity study, and other data summaries prepared by John Doherty; and tables and statistical analyses prepared by Bernice Fisher. The material reviewed is attached to the File Copy of this report.

C. Background Information:

Triphenyltin hydroxide is used to control fungal diseases on potatoes, sugar beets, peanuts, pecans, carrots, soybeans, rice, cocoa, and coffee. The Tox. Chem. No. is 896E.

Structure of Triphenyltin hydroxide:



Triphenyltin Hydroxide

D. Evaluation of Carcinogenicity Evidence for TPTH.

1. Rat Oncogenicity Study

Reference: Tennekes, H., K. Horst, H. Luetkemeier, W. Vogel, O. Vogel, B. Schlotke, H.A. Bhlers, E. Muller, Ch. Terrier. April 18, 1989. TPTH Technical (Code: HOE 029664 of ZD97 0004) Chronic toxicity/oncogenicity 104-week feeding study in rats. Unpublished report number 046980 by Research Consulting Company (RCC), Itingen, Switzerland. Submitted by Hoechst Celanese Corporation, Somerville, N.J. MRID No. 410857-02.

Triphenyltin hydroxide (TPTH; 97% a.i.) was administered via the diet to groups of 70 male and 70 female Wistar rats (KFM-Han; outbred) at dose levels of 0, 5, 20, and 80 ppm for 104 weeks. At the end of 12 months, 10 animals/sex/dose group were sacrificed.

a. Discussion of tumor data.

Female rats had significant differences in the pair-wise comparison of controls and both the mid (20 ppm) and the high (80 ppm) dose groups in pituitary gland adenomas (Table 1). Male rats had a significant dose-related increase in Leydig cell tumors with incremental doses of TPTH (Table 2). These tumors also were significantly different in the pair-wise comparison of the control and high (80 ppm) dose group. No statistically significant changes occurred in pituitary gland adenomas in male rats with increasing doses of TPTH.

In female rats, fatal tumor analysis indicated a significant difference in pituitary gland adenomas in the pair-wise comparison of controls with mid (20 ppm) dose and high (80ppm) dose groups (Table 1). Pituitary gland adenomas in the female rats also had a dose-related trend, of borderline statistical significance (p=.054).

Table 1. TPTH - Female Rat, Pituitary Gland Adenoma Tumor Rates[+] and Statistical Test Results (p values).

	Dose(ppm)			
	0	5.00	20.00	80.00
Test Animal Deaths on Study	14/19 (74)	20/29 (69)	33[a]/38 (87)	43/46 (93)
p[++]=	0.054	0.028(n)	0.006**	0.010**
Terminal Sacrifice	24/40 (60)	16/28 (57)	10/18 (56)	11/13 (85)
Total	38/59 (64)	36/57 (63)	43/56 (77)	54/58 (93)
p[+++] =	0.0001**	0.521	0.106	0.0003**

[+] Number of tumor bearing animals/ Number of animals examined.

[++] Results of fatal tumor analysis [Generalized K/W Analysis] applied to animals dying on study.

[+++] Results from Cochran-Armitage Trend test and Fisher Exact test.

(n) Negative change from control.

[a] First adenoma observed at week 48, dose 20ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

if * then p<.05 and if ** then p<.01.

Table 2. TPTH - Male Rat, Tumor Rates+ and Statistical Test Results (p values)

	Dose(ppm)			
Tumors	0	5.00	20.00	80.00
Testes Leydig(%) Cell	1/68 (1)	5/69 (7)	3/70 (4)	11[a]/70 (16)
p++=	0.001**	0.108	0.321	0.003**
Pituitary Gland Adenoma(%)	10/19 (53)	7/13 (54)	12[b]/19 (63)	10/13 (77)
p+++=	0.151	0.620	0.428	0.320

+ Number of tumor bearing animals/ Number of animals at risk, excluding those that died before observation of the first tumor.

++ Cochran-Armitage Trend test and Fisher Exact test results

+++ Results of fatal tumor analysis [Cox's test] applied to animals dying on study.

a First Leydig cell tumor at week 53, dose 80ppm.
b First pituitary gland adenoma at week 53, dose 20ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

if * then p<.05 and if ** then p<.01.

The increased incidence of pituitary gland adenomas in female rats at 80 ppm of TPTH (i.e., 54/58 or 93%) exceeded historical incidences in eight other 104 to 130 week feeding studies performed in female rats of the same strain in the testing laboratory for the years 1985-1988 (range of 51-79%). The increased incidence of pituitary gland adenomas at 20 ppm TPTH (i.e., 42/56 or 77%) exceeded historical control incidences in six of the eight 104-130 week feeding studies.

In female rats there was a significant dose-related increasing trend in mortality (Table 3). Also, the pair-wise comparison with control and the low (5 ppm), and mid (20 ppm), and high high (80 ppm) dose groups resulted in significant differences in mortality. Since the fatal tumor analysis of pituitary gland adenomas in female rats excluded data from the interim and from the final sacrifice groups, data (Table 4) were summarized to show the total distribution of these tumors by different time periods, and also the totals observed throughout the study. The Peer Review Committee agreed that the study pathologist's report, that the deaths were related to the pituitary tumors, should be accepted.

Table 3. TPTH - Female Rat Study, Morality Rates+ and Cox or Generalized K/W Test Results

	Weeks					
Dose(ppm)	1-26	27-52	53[a]	53-77	78-104[b]	Total
0	0/70	1/70	10/10	1/59	17/58	19/60(32)**
5	2/70	1/68	10/10	7/57	20/50	30/60(50)*
20	3/70	3/67	10/10	5/54	29/49	40/60(67)**
80	2/70	1/68	9/9	10/58	35/48	48/61(79)**

+ Number of animals that died during interval/ Number of animals alive at the beginning of the interval.

( ) percent

a Interim sacrifice
b Final sacrifice at weeks 105-107

Note: Time intervals selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

if * then p<.05 and if ** then p<.01.

Table 4. TPTH - Wistar Rats, Female Pituitary Adenomas by Selected Time Periods

Dose (ppm)	Weeks 1-52	53-IS	53-77	78-104	FS	Total
0	0/1(0)	1/10(10)	1/1(100)	13/17(76)	24/40(60)	39/70(56)
5	0/3(0)	1/10(10)	5/7(71)	15/20(75)	16/28(57)	37/70(53)
20	1/6(17)	0/10(0)	5/5(100)	27/29(93)	10/18(56)	43/70(61)
80	0/3(0)	2/9(22)	10/10(100)	33/35(94)	11/13(85)	56/70(80)

+ Number of tumor bearing animals/Number of animals that died & were examined during this time period.

( ) percent

IS Interim Sacrifice
FS Final Sacrifice

b. Consideration of Adequate Dosing for Assessment of Carcinogenic Potential.

The highest adequate dose is considered to have been attained, based on decreases in body weight (-21.1% for males; -16.7% for females). Tumor-related deaths were observed in females, and non-neoplastic pathological changes (i.e., cystoid change in pars intermedia of pituitary gland and tubular atrophy of the testes) were noted in the organs where tumor incidence was increased by treatment. The Peer Review Committee noted that the tubular atrophy lesion could be secondary to tumor growth.

2. Mouse oncogenicity study.

Reference: Tennekes H., K. Horst, H. Luetkemeier, W. Vogel, O. Vogel, J. Armstrong, H.A. Bhiers, E. Muller, Ch. Terrier. April 14, 1989. TPTH Technical (Code HOE 029666 of ZD97 0004) oncogenicity 80 week study in mice. Unpublished report number 047002. Prepared by Research and Consulting Company (RCC). Submitted by Hoechst Celanese Corporation, Somerville, N.J. MRID No. 410857-01.

TPTH (97.2% a.i) was administered via the diet to groups of 50 male and 50 female NMRI mice (KFD-Han) at dose levels of 0, 5, 20, and 80 ppm for 80 weeks.

a. Discussion of tumor data.

In female mice, combined hepatocellular tumors (adenomas and/or carcinomas), hepatocellular carcinomas only, and hepatocellular adenomas only, each had a significantly increasing trend with dose increments of TPTH. Both the adenoma rates and the combined (adenoma and/or carcinoma) rates were significantly different in the pair-wise comparison of the control and the highest (80 ppm) dose group (Table 5).

Table 5. TPTH - Female Mice, Hepatocellular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose(ppm)			
Tumor	0	5.00	20.00	80.00
Adenoma	0/48	0/48	0/49	8[a]/44
(%)	(0)	(0)	(0)	(18)
p=	0.000**	1.000	1.000	0.002**
Carcinoma	0/48	0/48	0/49	3[b]/44
(%)	(0)	(0)	(0)	(7)
p=	0.001**	1.000	1.000	0.106
Both	0/48	0/48	0/49	11/44
(%)	(0)	(0)	(0)	(25)
p=	0.000**	1.000	1.000	0.000**

+ Number of tumor bearing animals/ Number of animals at risk, excluding those that died before 52 weeks.

a First adenoma observed at week 59, dose 80ppm.
b First carcinoma observed at week 72, dose 80ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

if * then p<.05 and if ** then p<.01.

In male mice, combined hepatocellular tumors (adenomas and/or carcinomas) had a significantly increasing trend with dose increments of TPTH, primarily due to the significantly increasing trend in the adenomas. Both the adenoma rates and the combined rates (adenomas and/or carcinomas) were significantly different in the pair-wise comparison of the control and the highest (80 ppm) dose group. The adenoma rate also was significantly different in the pair-wise comparison of the control and the mid (20 ppm) dose group (Table 6).

Table 6. TPTH - Male Mice, Hepatocellular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose(ppm)			
Tumors	0	5.00	20.00	80.00
Adenoma	5/49	10[a]/48	13/47	15/48
(%)	(10)	(21)	(28)	(31)
p=	0.017*	0.122	0.026*	0.010**
Carcinoma	2[b]/49	1/48	0/47	3/48
(%)	(4)	(2)	(0)	(6)
p=	0.126	0.508(n)	0.258(n)	0.490
Both	7/49	11/48	13/47	18/48
(%)	(14)	(23)	(28)	(38)
p=	0.007**	0.203	0.086	0.008**

+ Number of tumor bearing animals/ Number of animals at risk (excluding those that died before 52 weeks).

(n) negative change from control.

a First adenoma observed at week 55, dose 5ppm
b First carcinoma observed at week 82, dose 0ppm

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

if * then p<.05 and if ** then p<.01.

The increased incidence of hepatocellular adenomas seen in male mice at 80 ppm (i.e., 15/50 or 30%) and in female mice at 80 ppm (i.e., 8/50 or 16%) exceeded historical control incidences in twelve other studies performed in male and female mice of the same strain in the testing laboratories for the years 1983-1988 (range for males: 0-16%; range for females: 0-4%). The Peer Review Committee noted that historical control incidences for hepatocellular adenomas in the 3/12 studies that were run for 79-80 weeks was 0% in females, and ranged from 0-12% in males.

The Peer Review Committee also noted that the appearance of hepatocellular carcinomas in female NMRI mice is a very uncommon event; and this was supported by historical data presented which showed that in 11/12 studies cited the incidence of hepatocellular carcinomas was 0%, and was 2% in the remaining study.

b. Considerations of Adequate Dosing for Assessment of Oncogenic Potential.

In female mice there was a statistically significant increasing trend in mortality, but no significant differences between the control and any dose group. In male mice, there was no statistical evidence of dose related mortality, or in the pair-wise comparisons of control and any dose group. The highest adequate dose was considered to have been attained because the following were noted in the high (80 ppm) dose group: significantly decreased body terminal body weight gains in male (-12%) and in female (-15%) mice; decreased immunoglobulin classes in male and female mice (IgG = -34% in males, -54% in females; IgA = -28% in males, -24% in females; IgM = -40% in males, - 27% in females; signs of "ill-health", including "ruffled fur" in females, increase in absolute liver weight (+24%) in males, and increased liver:body weight (+40.3% in males, +34% in females), and increased liver:brain weight (+24.1% in males, +17.6% in females).

E. Additional Toxicology Data on:

1. Carcinogenicity:

A study was done (NCI/Litton Bionetics; Study #78-1394, Published, 1978) with $B_6C_3F_1$ mice and Fischer 344 rats dosed via the diet with TPTH at 0, 37.5, and 75 ppm for 17 months, followed by a 6 month period during which TPTH was withdrawn from the diet. There were 50 animals/strain/sex/dose group, and 20 animals/strain/sex in the control group. The study was classified as Supplementary because only summary data were provided in the final report. The pertinent lesions observed in the rat were as follows (Data reported as number of animals showing lesion and percent):

		Dose group (ppm TPTH)		
Lesion	Sex	0	37.5	75
Chromophobe adenoma of pituitary gland	M	2/20(10%)	5/48(10%)	9/46(20%)
	F	7/17(41%)	32/47(68%)	24/49(53%)
Interstitial-cell tumor of testis	M	17/19(89%)	46/50(92%)	49/50(98%)

No Leydig cell adenomas were reported in male rats. The data from the mouse study did not reveal any dose dependent increases in hepatocellular tumors.

2. Metabolism.

Studies (EPA Accession Nos. 400294-05, -06, and-07) with ^{14}C-ring labelled TPTH show that most of the ^{14}C is excreted in the feces. ^{14}C excreted in the urine was identified mostly as benzene metabolites. A more recent study (EPA Accession No. 413091-01 and-02) with ^{113}Sn-labeled TPTH showed that about 25% can be absorbed from the gastrointestinal tract of male rats and about 12% is absorbed from the gastrointestinal tract of female rats. The label did not selectively accumulate in the testes, pituitary glands, or the liver. The liver was among the organs showing the highest accumulation of the label.

3. Structure:Activity Relationships.

A carcinogenicity study conducted in rats by the World Health Organization with tributyltin hydroxide was summarized to report that increases in adrenal and pituitary gland tumors were observed.

4. Developmental toxicity.

TPTH shows developmental toxicity in rabbits (EPA Accession No. 401048-01), and has a NOEL of 0.1 mg/kg/day for maternal toxicity effects in dams. A NOEL of 0.9 mg/kg/day was determined for resorptions. A rat multigeneration study (EPA Accession No. 264667-264676) gave a NOEL for TPTH of 0.25 mg/kg/day, based on decreased survival of pups and smaller litter size. Developmental toxicity issues with TPTH will be peer-reviewed separately.

5. Mutagenicity.

The submitted studies present a database that satisfies the requirements for mutagenicity testing. The weight-of-the-evidence suggests that there is little support for a mutagenicity concern. The major positive finding is with the cultured human lymphocyte assay. However, the bulk of the in vivo data suggests that there may not be a large concern when test animals are exposed to TPTH. The submitted studies in the three mutagenicity categories are summarized as follows:

Study	Results
1. Gene Mutation Tests	
Bacterial-reverse mutation in S. typhimurium and E. coli Huntington Re. Center #450/ 81A, July , 1981 (EPA Acces. sion No. 071368).	Not mutagenic in E. coli of S. typhimurium with and without metabolic activation.
Gene mutation in E. pombe. Inst. di Ricerche Biom. Antione Marxer, #M 889 August 20, 1985 (EPA Accession No. 259345).	Negative up to and including cytotoxic levels.
Mouse Lymphoma forward mutation assay. Litton Bionetics (Netherlands) #E09406, August 1985 (EPA Accession No. 259345).	Borderline positive at 250 and 300 ng/ml in the presence of S-9 mix. Negative in the absence at up to and including cytotoxic levels.
2. Structural Chromosome Aberration Tests	
Mouse micronucleus (in vivo) RCC (Switzerland) #049552, August 5, 1985 (EPA Accession No. 259345).	Negative at up to and including 77% of the LD50, a dose level showing signs of toxicity.
In vivo cytogenetic test in bone marrow cells of the Chinese hamster. Pharma (Germany) #86-1104 (EPA Accession No. 403711-02)	Negative at dose levels up to and including 80 mg/kg. Assessed at 12, 24 and 48 hours post dosing.
Human lymphocyte cytogenetics (in vitro) Microtest Research Ltd.; #HOF2/HLC/KF17/HL1 (EPA Accession No. 259345; HED Document No. 006589)	Positive dose-response without activation to 1 ug/ml; Positive with activation to 2 ug/ml.
3. Tests for Other Genotoxic Effects	
Gene conversion in S. cerevisiae D4. Inst. di Richerhe Biom. Antione Marxer. #M890, October 24, 1985 (EPA Accession No. 260962)	Negative up to and including 5 ug/ml nonactivation system and 15 ug/ml with the S-activation system.
Unscheduled DNA synthesis in rat primary hepatocytes. Litton Bionetics. #20991, October, 1985 (EPA Accession No. 260962).	Negative up to and including dose levels that are cytotoxic (i.e., 0.5 ug/ml).

6. Immunotoxicity.

Reports in the scientific literature show that organotin compounds, including TPTH, can suppress cell-mediated immune response in the rat (e.g., Vos J., et al., 1984, Toxicology, 29:325-336). Subchronic and chronic feeding studies submitted to the Agency indicate also that TPTH can cause reductions in immunoglobulin classes (i.e., IgG, IgM, and IgA). A study currently is being performed in mice and in rats to establish a NOEL for TPTH immunotoxicity.

7. Acute, Subchronic, Chronic Effects.

The acute oral LD_{50} values in rats are 165 mg/kg for males and 156 mg/kg for females (EPA Accession No. 071364). Depending on the conditions of the study, the acute dermal toxicity LD_{50} values in rabbits was 3000 mg/kg (range: 1820-4950 mg/kg; EPA Accession No. 0083560) or was 127 mg/kg (EPA Accession No. 071364). The acute inhalation LC_{50} value for rats was 60.3 mg/mm^3, with some deaths being delayed (EPA Accession No. 071364). TPTH is corrosive to the eye (EPA Accession No. 071364).

Toxic effects observed in a 90-day rat feeding study (EPA Accession No. 261754) included decreased immunoglobulin levels at the lowest dose tested (4 ppm), and decreased food consumption and body weight gain at 100 ppm. Toxic effects observed in a 90-day mouse feeding study (EPA Accession No. 261753) included decreased immunoglobulin levels (again, at 4ppm; the lowest dose tested), and increased liver weights at 100 ppm.

In a 1-year dog study (EPA Accession No. 402855-01), the NOEL was >18 ppm (the highest dose tested).

F. Weight of Evidence Considerations.

The Committee considered the following facts regarding the toxicology data on TPTH to be of importance in a weight-of-evidence determination of carcinogenicity potential.

1. TPTH when administered via the diet to female Wistar rats at doses of 0, 5, 20, and 80 ppm for two years was associated with significant increases for pituitary gland adenomas in the pair-wise comparison of control versus both the mid (20 ppm) and the high (80 ppm) dose groups. Fatal tumor analysis showed that pituitary gland adenomas in female rats also had a dose-related trend, of borderline statistical significance ($p<.054$).

2. In female rats there was a significant dose-related increasing trend in mortality. Also, the pair-wise comparison with control and the low, and mid, and high dose groups resulted in significant differences.

3. The pathologists report stated that the pituitary gland adenomas were fatal tumors.

4. The increased incidence in female rats of pituitary gland adenomas exceeded historical control incidences in eight other studies performed in female rats of the same strain in the testing laboratory for the years 1985-1988.

5. In male Wistar rats dosed via the diet with TPTH at 0, 5, 20 and 80 ppm for two years, there were no statistically significant changes in pituitary gland adenomas with increasing doses of TPTH.

6. There was a significant dose-related increase in Leydig cell tumors in testes of male rats. Leydig cell tumors were also significantly different in the pair-wise comparison of the control and high (80 ppm) dose group.

7. For the rat study, the highest adequate dose was attained. The dosing was considered sufficient for assessment of oncogenic potential.

8. TPTH when administered via the diet to male and female NMRI mice at doses of 0, 5, 20, and 80 ppm for 80 weeks was associated with a significant increasing dose-related trend in hepatocellular adenomas and in combined hepatocellular (adenomas and/or carcinomas) tumors in males and in females. Female mice also had an increasing dose-related trend in hepatocellular carcinomas.

9. Male and female mice had a significant difference in hepatocellular adenomas and combined hepatocellular (adenomas and/or carcinomas) tumors in the pair-wise comparison of controls with the high (80 ppm) dose group. Male mice also had a significant difference in adenomas in the pair-wise comparison of controls with the mid (20 ppm) dose group.

10. The increased incidence of hepatocellular adenomas seen in male and in female mice at 80 ppm of TPTH exceeded the historical control incidences in twelve other studies performed in mice of the same strain in the testing laboratory during the years 1983-1988.

11. The appearance of hepatocellular carcinomas in female NMRI mice is a very uncommon event; eleven of the twelve cited historical control studies showed a 0% incidence, and the remaining study a 2% incidence.

12. For the mouse study the highest adequate dose was attained. The dosing was considered adequate for assessment of oncogenic potential.

G. Classification of Carcinogenic Potential.

Criteria contained in the EPA Guidelines [FR 51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee concluded that the data available provided evidence to classify TPTH as a Group B2 carcinogen ("probable human carcinogen"). This was based on: the significant increases in fatal pituitary gland adenomas in female Wistar rats and

Leydig cell tumors in male Wistar rats; and, the significant increases in male and female NMRI mice of hepatocellular adenomas and combined hepatocellular (adenoma and/or carcinoma) tumors and, in female mice, a positive trend for hepatocellular carcinomas. There was little concern for genotoxicity of TPTH.

The Committee considered that quantification of carcinogenic risk for TPTH is appropriate. In addition to the above, the following factors were taken into consideration: hepatocellular carcinomas are considered as very uncommon in female NMRI mice; tumor incidences were significantly increased at relatively low dose levels of TPTH; and, evidence from scientific literature and from submitted sub-chronic and chronic studies indicate that TPTH exerts adverse effects on cell-mediated and humoral components of the rodent immune system.

The Peer Review Committee considered the merits of basing the quantification of risk on the rat pituitary adenoma data taking into account both the interim and final sacrifice animal data, versus basing the risk quantification on the mouse combined hepatocellular (adenoma and/or carcinoma) tumor data. It was concluded that the risk quantification should be performed independently on the pituitary tumor data and on the hepatocellular tumor data.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D C 20460

MEMORANDUM

SUBJECT: Revised Peer Review Committee Document for TPTH

TO: Esther Rinde, Ph.D.
SACB, HED (H7509C)

FROM: Roy D. Sjoblad, Ph.D.
SACB, HED (H7509C)

Attached is the revised Peer Review Document for carcinogenicity evaluation of TPTH. comments were received from R. Engler, J. Quest, R. Levy, K. Dearfield, C. Baetke, R. Gardner, and J. Doherty. All major comments and most minor comments were taken into consideration and incorporated into the revised document. Among the major revisions from the original Draft include: A revised version of Table 1; a re-presentation of the TPTH tumor data as related to historical control studies; upgrading of the Metabolism section to take into account a recently reviewed new study; inclusion of an additional mutagenicity study, and restatement of the Mutagenicity section; and correction of a certain few, but egregious, errors I made in the initial Draft.

None of the revisions affect the conclusions or recommendations of the initial Draft. Please include this information when the revised document is circulated in the next cycle, in that I believe it might be of assistance for those commenters who wish to check on how their comments were incorporated.

Also, this Memorandum could serve as notice of appreciation for those who did render careful attention to the Draft, and in addition, for the efforts of H. Pettigrew and B. Fisher who thoughtfully - and with patience - clarified for me some of the key statistical issues, and likewise for R. Gardner, who clarified certain of the esoteric biological issues.

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

APR 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Subject: TPTH (Triphenyltin hydroxide)- Quantitative Risk Assessment based on Dietary Studies in Mice and Rats II

caswell no. 896E

From: Hugh Pettigrew, Ph.D., Statistician 4/4/91
Bernice Fisher, Biostatistician 4/4/91
Science Support and Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: Larry Dorsey
Chemical Manager for TPTH
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Thru: Esther Rinde, Acting Head 4/4/91
Science Support and Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

And: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Summary

The single most appropriate estimate of the unit risk, Q_1^*, of TPTH in human equivalents is

$$Q_1^* = 2.8 \times 10^0 (mg/kg/day)^{-1}$$

This is based on fatal pituitary gland adenomas in female Wistar rats.

Background

The Peer Review Committee meeting of 11/29/89 on TPTH concluded that the chemical compound should be classified as a [B_2] carcinogen. They also recommended that the unit risk, Q_1^*, should

be estimated independently from four data sets based on four different and statistically significant tumor rate increases due to dietary exposures of TPTH (0,5,20, and 80 ppm dose levels). The tumors were male and female mouse hepatocellular adenomas and/or carcinomas, and rat female total pituitary gland adenomas and also only female fatal pituitary gland adenomas.

Dose-Response Analysis

The calculations of the unit risk, Q_1^*, were performed using a computer program provided by K. Crump (TOX_RISK Version 3). The Global multi-stage model was fit to the hepatocellular tumor data (adenomas and/or carcinomas) in male and female mice, since there were no significant dose related mortality differences in either sex. Calculations of unit risk based on total and fatal pituitary gland adenomas in female rats were based on the multistage Weibull (time to tumor) model, because there was significant differential mortality. The resulting four estimates of unit risk were given in a previous memorandum (TPTH (Triphenyltin hydroxide)- Quantitative Risk Assessment based on Dietary Studies in Mice and Rats, B. Fisher and H. Pettigrew, 10/4/90.)

The estimates of Q_1^* were as follows:

1. NMRI Mouse, male hepatocellular (adenoma and/or carcinoma) tumors:
   $Q_1^* = 6.2 \times 10^{-1} (mg/kg/day)^{-1}$
2. NMRI Mouse, female hepatocellular (adenoma and/or carcinoma) tumors:
   $Q_1^* = 1.1 \times 10^{-1} (mg/kg/day)^{-1}$
3. Wistar Rat, female pituitary gland adenomas (including terminal sacrifice):
   $Q_1^* = 2.9 \times 10^{0} (mg/kg/day)^{-1}$
4. Wistar Rat, female fatal pituitary gland adenomas (excluding sacrificed animals):
   $Q_1^* = 2.8 \times 10^{0} (mg/kg/day)^{-1}$

The more conservative estimates of human risk can be seen to be those based on the female rat pituitary tumors. Of the two methods of analysis based on the female rat, the estimates of risk are essentially the same. The method based on excluding tumors found in animals terminally sacrificed is preferable. In this method of analysis, terminally sacrificed animals are treated as being withdrawn from the study alive before their potential "death from fatal tumor" is observed.

Reviewer's Peer Review Package for 1st Meeting

11/14/89

FILE COPY

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

NOV 16 1989

MEMORANDUM

SUBJECT: Peer Review on Triphenyltin Hydroxide (TPTH) PESTICIDES AND TOXIC SUBSTANCES

FROM: Esther Rinde, Ph.D. E.R.
Manager, ONCO Peer Review
Health Effects Division (TS-769c)

TO: Addressees

Attached for your review is a package on TPTH prepared by Dr.John Doherty

A meeting to consider the classification of TPTH is scheduled for 11/29/89 at 10:00 in Room 821, CM2.

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
B. Beliles
K. Baetcke
M. Van Gemert
M. Copley
J. Quest
K. Dearfield
R. Levy
W. Sette
G. Ghali
B. Fisher
J. Du
Y. Woo
R. Sjoblad
J. Doherty
R. Gardner

2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

NOV 5, 1989

OFFICE OF
PESTICIDES AND

MEMORANDUM

SUBJECT: Peer Review of Carcinogenicity of Triphenyltin Hydroxide.

TOX CHEM No.: 896E

FROM: John Doherty 11/15/89
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

TO: Esther Rinde
Manager, Peer Review for Carcinogenicity
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Karl Baetcke 11/15/89
Chief, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Background

Triphenyltin hydroxide (TPTH) is an organotin fungicide. The data base was reviewed and a Registration Standard prepared. In response to the data gaps indicated in the Registration Standard the registrant has submitted rat and mouse carcinogenicity studies. Review of these studies (refer to memo from J. Doherty for EPA Reg. No. 8340-17, September 27, 1989) indicated that TPTH is carcinogenic in these species. In particular, the following organs have been implicated as carcinogenic targets for TPTH.

Pituitary. In female rats as indicated by adenomas.

Testis. In rats as indicated by Leydig cell adenomas.

Liver. In mice as indicated by both adenomas (males and females) and carcinomas (females only).

In both the rat pituitary and testis, there is significant nonneoplastic pathology at dose levels showing increased incidence of tumors.

3

It is requested that TPTH be reviewed by the Peer Review Committee and a recommendation made for its classification for carcinogenicity group and as to whether or not quantitative risk assessments need to be made for the various uses and tolerances associated with this pesticide.

The following information is being provided in this package.

Part A. Summary of the Neoplastic Findings and Overview of the Chronic Feeding and Carcinogenicity Studies. Including a discussion of the carcinogenicity categorization parameters.

Part B. Topical Discussions of Metabolism, Structure Activity Relationships, Developmental Toxicity, Mutagenicity, Immunotoxicity and Endocrine Toxicity of TPTH.

Important Note: The statistical procedures used in Part A of this document are based on the total number of animals actually examined without adjustment for time of death.

A separate report is being prepared by Ms. Bernice Fisher which includes adjustments for time to death and analyses based on the number of animals at risk. The mid dose male mouse liver and the mid dose female rat pituitary data show statistical significance when assessed by Ms. Fisher's analysis but these groups did not show statistical significance when assessed by other methods (see p. 4 for rat pituitary data and p. 7 for mouse liver data).

Part A. Summary of Neoplastic Findings and Overview of the Chronic Feeding and Carcinogenicity Studies. The following five carcinogenicity studies with TPTH have been reviewed by TB-I:

Rat Studies

1. NCI/Litton Bionetics (Study 78-1394, Published, 1978)

In this study three groups of Fischer 344 rats were dosed with either 0, 37.5 or 75 ppm of TPTH in their diets for 17 months and allowed an additional six months without TPTH in their diets before sacrifice. They were not dosed for the 2 year period. There were initially 50 rats of each sex in each group dosed with TPTH and 20 rats of each sex in the control groups.

The following table illustrates the findings in the pituitary and testis as reported in this study.

Lesion		Test Group Control	37.5ppm	75 ppm
Pituitary	N[1]	20/17	48/47	46/49
Chromophobe adenoma	M	2(10%)	5(10%)	9(20%)
	F	7(41%)	32(68%)	24(53%)
Testis	N	19	50	50
Interstitial-cell tumor[2]	M	17(89%)	46(92%)	49(98%)

[1] N = number of males/females examined.
[2] Note: No Leydig-cell tumors reported.

This study is classified as SUPPLEMENTARY because of the summary nature of the final report, no individual animal data were included, and the rats were not dosed for a 2 year period. This study is not regarded by TB-I as demonstrating carcinogenic effects of TPTH.

[redacted]

2. RCC Study (Study #046980, April 18, 1989)

In this study four groups of 70 Wistar male and 70

female rats were dosed with TPTH at either 0, 5, 20, or 80 ppm for two years. The females at all dose levels were reported to have increased deaths allegedly due to pituitary tumors. The following two tables illustrate the pathological findings in this study which indicate that the pituitary and testis are both carcinogenic and non-carcinogenic target organs for TPTH.

Table 1. Neoplastic and non-neoplastic findings in the pituitary.

Lesion		Dose Level Control	5 ppm	20 ppm	80 ppm
A. Macroscopic	N	70/70	70/70	70/70	70/70
Nodules	M	17(24)	16(23)	21(30)	18(26)
	F	21(30)	26(37)	33(47)	47(67)
Compressing Brain	M	11(16)	11(16)	14(20)	8(11)
	F	15(21)	15(21)	26(37)	31(44)
Deaths due to pituitary tumor[2]	F	8/19 (42)	17/29 (57)	24/38 (63)	34/47 (72)
B. Microscopic					
Pars Intermedia	N	53/49	55/41	56/36	57/46
hyperplasia	M	-- --	--	12(21)***	
cystoid change[5]	M	8(15)	11(20)	29(52)***	38(67)***
	F	10(20)	8(20)	6(17)	28(61)***
Pituitary Gland	N	68/69	68/67	67/66	69/68
Adenoma (Total)	M	24(35)	20(29)	37(55)	26(46)
	F	39(57)	37(55)	43(65)	56(82)***
Adenoma (among decedents)[2]	F[3]	14/19 (74)	20/29 (69)	33/38 (87)	43/46 (95)

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

[1] N refers to the number of animals observed (males/females, when data for both sexes are presented).

[2] Only data for females are presented. In this row, the numerator is the number of rats that were judged by the testing laboratory to have died as a result of having a pituitary tumor. The denominator is the number of unscheduled deaths.

[3] In this row the numerator is the number of rats with a

pituitary adenoma and the denominator is the number of unscheduled deaths.

[T] The trend is statistically significant, *** P < 0.001

[5] The "cystoid change" was described by the pathologists as "characterized by the occurrence of round or cleft-like spaces. These spaces were occasionally filled with serous fluid or blood constituents".

A carcinogenic 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 Wistar female rats is 40-79% for 13 studies (mean 58.4 ± 12.6 %) conducted in 1985-1988. The high dose female group in this study (82.4 to 93% incidence) is clearly in excess of the historical control range and mean.

The mid dose female group was also demonstrated to be statistically significantly increased by Ms. Fisher's statistical tests. The percentage incidence for this group (65% incidence) was, however, within the historical control range.

Table 2. Neoplastic and non-neoplastic findings in the testis.

Lesion		Dose Level (%) Control	5 ppm	20 ppm	80 ppm
Non-neoplastic	N	60	59	60	60
Leydig Cell Hyperplasia		5(8)	6(10)	11(18)	24(40)***[1]
Tubular Atrophy		6(10)	9(15)	10(17)	17(28)** [1]
Neoplastic					
Leydig Cell Tumors		1(2)	5(9)	3(5)	10(17)**[1]

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

Leydig Cell tumors were reported to have a range in percentage occurrence of 0-8% (mean 3.2 ± 2.9%) 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 (Leydig Cell hyperplasia and tubular atrophy) 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.

For both target organs (pituitary and testis) in the rat there is significant non-neoplastic pathology at the same or lower dose levels than there is neoplastic pathology. This raises the possibility that the apparent carcinogenic effects in these organs are secondary to some other effect.

Other systemic effects in this study included: decreases in immunoglobulins; decreases in body weight particularly noticeable after the first year, final body weights for the mid dose group males (-7.7%), and high dose group males (-21.1%) and females (-16.7%) were statistically significantly different from controls; bile duct proliferation and portal sclerosis; sciatic nerve degeneration and skeletal muscle atrophy. The Maximum Tolerated Dose is considered to have been attained and possibly exceeded based on decreases in body weight, deaths in females and non-neoplastic pathological changes in the potential oncogenic target organs.

Mouse Studies

3. Cannon Laboratories (Study #6E-725, August 28, 1978).

This study was conducted by the Cannon Laboratories. The study raised the question of possible lesions in the uterus described as "endometrial hyperplasia" being a response to the TPTH. In the study report this lesion type was listed under neoplastic findings.

TB requested that the slides be reexamined and the lesion be further defined as being neoplastic or otherwise. The study was subsequently determined to be INVALID by TB and of no usefulness in evaluating the carcinogenic potential in mice because the registrant was unable to provide a satisfactory account of the tissue slides and other records for this study.

4. NCI/Litton Bionetics (Study #78-1394, Published, 1978)

B6C3F1 mice were dosed with 0, 37.5 and 75 ppm of TPTH for 17 months and allowed to live an additional 6 months without TPTH in their diet. They were not dosed for a two year period. There

were initially 50 mice per sex per dose group in the treated groups and 20 mice per sex in the control groups.

No dose dependent increases in tumors were reported. The neoplastic findings in the liver for this study are illustrated in the following table.

		Test Dose Group		
Lesion		Control	37.5 ppm	75 ppm
Liver	N*	19/18	44/42	44/48
Hepatocellular adenoma	M	2(11%)	5(11%)	3(7%)
	F	1(6%)	2(5%)	3(6%)
Hepatocellular carcinoma	M	3(16%)	1(2%)	7(16%)
	F	0	0	1(2%)

*N number of males/number of females examined.

The study was classified as SUPPLEMENTARY because of the summary nature of the final report. No individual animal data were presented. This study is not regarded by TB-I as demonstrating carcinogenic effects of TPTH.

5. RCC Study (study #047002, April 14, 1989)

In this study four groups of 50 male and 50 female mice (NMRI strain) were dosed with either 0, 5, 20 or 80 ppm of TPTH for 104 weeks. The following table illustrates the findings in the liver in this study.

		Dose Group			
Lesion		Control	5 ppm	20 ppm	80 ppm
	No.[2/]	50/50	50/50	50/50	50/50
NODULES[1/]	M	4(8)	9(18)	8(16)	16(32)
	F	2(4)	0	1(2)	6(12)
Neoplastic Findings					
	No.	49/50	50/50	50/50	50/50
HEPATOCELLULAR ADENOMA	M	6(12)	10(20)	13(26)	16(32)*
	F	0	0	0	9(18)***

HEPATOCELLULAR	M	2(4)	1(2)	0	3(6)
CARCINOMA		0	0	0	3(6)NS
Non-Neoplastic Findings					
NODULAR	M	2(4)	1(2)	0	5(10)NS
HYPERPLASIA	F	1(2)	0	(2)	6(12)NS
"Fatty Change"	M	38(78)	43(86)	27(54)	9(18)
	F	34(68)	32(64)	22(44)	12(24)

* P < 0.05, *** P < 0.001, Fisher's Exact P, HED Computer
NS-Not significant.
[1/]Lesions written in capital letters show dose response increases.
[2/]The number of males and females examined is given as males/females.

One control male and one male and one female mouse in the high dose group has both an adenoma and carcinoma of the liver. Thus there were a total of 18 males and 11 females affected with liver tumors in the high dose group.

The historical control data provided by the testing laboratory (attached) indicate that the range for hepatocellular adenoma is 0-16% (mean 8.3 ± 5.3%) in males and 0-4% (mean 1 ± 1.4%) in females and for hepatocellular carcinoma it is 0-8% (mean 3.0 ± 2.5%) in males and 0-2% (mean 0.17 ± 0.58%) 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 mid dose male group was also demonstrated to be statistically significantly increased by Ms. Fisher's statistical test procedures.

The Maximum Tolerated Dose is considered to have been attained and may have been exceeded in females. The females in the high dose group developed signs of ill health and had a statistically significantly increased higher rate of deaths (52% in the high dose group vs 32% in the controls). Both male (-12%) and female (-15%) terminal body weights were decreased. The mid (+6-9%) and high (+10-12%) dose group females had increased body weights during the first 41-49 weeks of the study. After week 65 the high dose females showed weight decreases. Immunoglobulins IgA and IgM were reduced at all dose levels. Liver weight

(absolute or relative) was increased in the high dose group for both sexes. Kidney weight was reduced in the mid and high dose groups but without associated pathological changes.

Carcinogenicity Categorization Parameters.

1. Species. The rat (pituitary and testis) and mouse (liver) have been implicated to be carcinogenic targets for TPTH. Thus, TPTH is implicated to be carcinogenic in two species.

2. Sexes. Males developed increases in both testicular Leydig Cell tumors (in rats) and liver tumors (in mice). Females developed increases in both pituitary tumors (in rats) and liver tumors (in mice). Thus TPTH has been implicated to be carcinogenic in both sexes in two species.

3. Target Organs. The pituitary (rat), testis (rat) and liver (mouse) have been implicated to be target organs for TPTH. Thus, TPTH has been implicated as having multiple target sites for a carcinogenic effect.

4. Malignancy. The mouse liver in the female high dose group has 3 incidence of hepatocellular carcinoma and all other groups have zero incidence of this malignant tumor. Although incidence of carcinomas in female mice did not reach statistical significance, carcinomas in this strain of female mice are rare and may represent a progression from adenoma. See also point 6 below.

The Peer Review Committee will need to address the issue as to whether or not there is sufficient information to determine if TPTH treatment results in increases in malignancy.

There was no increase in malignant liver tumors in the male mice. There were no malignant tumors in either the pituitary or testis of rats.

5. Latency. The average week of death for the control, low, mid and high dose female rats with pituitary tumors was 101.6 $\pm$ 8.1, 93.4 $\pm$ 14.7, 90.8 $\pm$ 13.1, and 89.7 $\pm$ 14.8 respectively. These data imply a decease in the latency for development of pituitary tumors.

There was no evidence of a decrease in latency for the development of Leydig Cell tumors. Most of the rats with Leydig Cell tumors were in the terminal sacrifice groups although a single rat was in the interim sacrifice group.

In mice, the liver tumors were mostly in the terminal sacrifice groups for the high dose males and females thus no

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decrease in latency was evident.

6. Rare Tumors. Hepatocellular carcinoma in female NMRI strain is considered rare (0-2% range for 12 studies submitted by the registrant and in these studies there was only a single incidence of hepatocellular carcinoma in only one of these 12 studies). There was otherwise no evidence that TPTH treatment induced the formation of rare tumors presented in either the rat or mouse carcinogenicity studies.

PART B. Topical Discussions

1. Metabolism.

Studies with ^{14}C ring labelled TPTH demonstrate that most ^{14}C is excreted in the feces. ^{14}C excreted in the urine was identified as mostly benzene metabolites. Overall the data indicate that very little intact TPTH is absorbed from the gastro-intestinal tract.

The available metabolism data are considered SUPPLEMENTARY and an additional study using ^{113}Sn or ^{119}Sn has been requested to clarify the unresolved issues regarding the absorption and tissue distribution of TPTH.

2. Structure Activity Relationships.

There are no studies available with structural analogs of TPTH which TB-I has validated to compare the carcinogenic findings with TPTH as indicated above with.

The WHO conducted an carcinogenicity study in rats with tributyltin hydroxide and the Agency has a summary of the findings. This study was reported to indicate that tributyltin is associated with increases in adrenal and pituitary tumors.

3. Developmental Toxicity.

TPTH is currently considered to be a developmental toxin and has NOEL of 0.1 mg/kg/day based on maternal toxicity effects in the dams in a rabbit teratology study. Rabbits were also regarded as having a NOEL of 0.9 mg/kg/day for resorptions. The rat multi-generation reproduction study was demonstrated to have a NOEL of 0.25 mg/kg/day based on decreased survival of the pups and smaller litter size. TPTH is not, however, regarded as a frank teratogen.

4. Mutagenicity.

TPTH is considered to have an extensive mutagenicity data base including studies from categories I (gene mutation), II (structural chromosomal aberration) and III (other mechanism of mutagenicity). TPTH is not regarded as being mutagenic based on these studies. Refer to the Table summarizing the mutagenicity data base (attached).

5. Immunotoxicity.

Organotins are widely regarded as being immunotoxic (see Krajnc et al. Toxicology and Applied Pharmacology, 75, (363-386 1984). TPTH has been investigated for its effects on the immune system (Vos et al. Toxicology, 29, (325-336) 1984). Dr. Vos's conclusion was that "TPTH exposure suppresses the cell-mediated immunity without compromising the humoral immunity and the mononuclear phagocyte system". This study was published in the open scientific literature and was not validated.

Many of the studies submitted to the Agency and conducted with TPTH have indications that TPTH is also immunotoxic in rats, mice and guinea pigs. The evidence for this conclusion is supported by decreased blood cell counts and decreased immunoglobulins. A special study to assess for the immunotoxicity potential of TPTH has been requested. The results of this study are not expected to be submitted to EPA until June 1990.

In the absence of definitive and validated data on the immunotoxic potential of TPTH, it is premature to attempt to make causal relationships between immuotoxicity and the carcinogenicity effects noted in the rat and mouse studies.

6. Endocrine Toxicity.

Changes in hormonal levels have been reported for tributyl-tin hydroxide (Krajnc et al, Toxicology and Applied Pharmacology 75, 363-386 1984) such as decreases in insulin, thyroxin and thyrotropin and increases in leutinizing hormone. There are no validated data describing changes in hormone levels or other endocrine effects (except as above for the pituitary and testis) for TPTH.

The multi-generation reproduction study with TPTH did not indicate evidence for TPTH effects on hormones. The decreases in pup survival and pup body weight which are the effects at the LEL are too general to imply endocrine effects.

Additional data would be desireable to establish if the pathological changes of the pituitary and testis are preceded by changes in circulating hormone levels.

TABLE. Summary of mutagenicity studies submitted in support of TPTH and determined to be ACCEPTABLE.

Study	Results
1. Gene Mutation Tests	
Bacterial-reverse mutation in S. typhimurium and E. coli. Huntington Re. Center. #450/ 81A, July 21, 1981.	Not mutagenic in E. coli of S.typhimurium with and without metabolic activation. (Registration Standard).
Gene mutation in E. pombe. Inst. di Ricerche Biom. Antione Marxer, #M 889 August 20, 1985.	Negative up to and including cytotoxic levels. (Doherty memo 8/18/86).
Mouse lymphoma forward mutation assay. Litton Bionetics (Netherlands) #E-9406, August, 1985.	Borderline positive at 250 and 300 ng/ml in the presence of S-9 mix. Negative in the absence at up to and including cytotoxic levels. (Doherty memo 8/18/86)
2. Structural Chromosome Aberration Tests	
Mouse micronucleus (in vivo) RCC (Switzerland) #049552, August 5. 1985.	Negative at up to and including 77% of the $LD50_{50}$, a dose level showing signs of toxicity. (Doherty 8/18/86)
In vivo cytogenetic test in bone marrow cells of the Chinese hamster. Pharma (Germany) #86.1104.	Negative at dose levels up to and including 80 mg/kg. Assessed at 12, 24 and 48 hours post dosing. (Doherty
3. Tests for Other Geneotoxic Effects	
Gene conversion in S. cereisiae D4. Inst. di Richerhe Biom. Antione Marxer, #M890, October 24, 1985.	Negative up to and including 5 ug/ml nonactivation system and 15 ug/ml with the S-activation system. (Doherty 7/30/86).
Unscheduled DNA synthesis in rat primary hepatocytes. Litton Bionetics. #20991, October, 1985.	Negative up to and including dose levels that are cytotoxic (i.e. 0.5 ug/ml). (Doherty. 7/30/86).

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	RUO	49	48	7	24	14	50
006390 ◆	72 WEEK FEEDING	1985	RUO	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	RUO	49	48	-	-	0	0
006390 ◆	72 WEEK FEEDING	1985	RUO	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 M	ANIMALS EXAMINED F	ANIMALS WITH TUMORS M	ANIMALS WITH TUMORS F	INCIDENCE IN % M	INCIDENCE IN % F
005321	72 WEEK FEEDING	1985	JMA	50		-		0	
005321 •	72 WEEK FEEDING	1985	JMA	49		-		0	
006390	72 WEEK FEEDING	1985	RUO	50		3		6	
006390 •	72 WEEK FEEDING	1985	RUO	50		2		4	
017820	104 WEEK FEEDING	1986	HMW	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 M	ANIMALS EXAMINED F	ANIMALS WITH TUMORS M	ANIMALS WITH TUMORS F	INCIDENCE IN % M	INCIDENCE IN % F
005321	72 WEEK FEEDING	1985	JMA	50		-		0	
005321 •	72 WEEK FEEDING	1985	JMA	49		-		0	
006390	72 WEEK FEEDING	1985	RUO	50		-		0	
006390 •	72 WEEK FEEDING	1985	RUO	50		-		0	
017820	104 WEEK FEEDING	1986	HMW	50		-		0	
024300	104 WEEK FEEDING	1988	JMA	100		1		2	
027753	104 WEEK I. M.	1988	JMA	52		-		0	
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.

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

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:

"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 <u>females</u> 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	N	Control	5 ppm	20 ppm	80 ppm
		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).

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	92	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 M	ANIMALS EXAMINED F	ANIMALS WITH TUMORS M	ANIMALS WITH TUMORS F	INCIDENCE IN % M	INCIDENCE IN % 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 M	ANIMALS EXAMINED F	ANIMALS WITH TUMORS M	ANIMALS WITH TUMORS F	INCIDENCE IN % M	INCIDENCE IN % 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.

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 <u>decreases</u>; LEL = 80 ppm, liver weight <u>increases</u>.

8. <u>Gross Necropsy</u> - 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. <u>Histopathology</u> - 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. <u>Liver</u> - 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 <u>adenomas</u> in males (possibly all three dose levels) and females (high-dose group) and possibly hepatocellular <u>carcinomas</u> (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 <u>decreased</u> for males and <u>increased</u> 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. <u>Spleen</u> - 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 erythopoieses 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. <u>Adrenals</u> - 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.

Lesion		Control No.	5 ppm 50/50	5 ppm 13/27	20 ppm 29/29	80 ppm 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 both 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.

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

REVIEWER

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

005361

AUG 18 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 8340-17 - Review of Mutagenicity Studies with Triphenyltin Hydroxide (TPTH): DNA Damage in Human Lymphocytes, Gene Mutation in Schizosaccharomyces Pombe, Mouse Micronucleus Test, and Mouse Lymphoma Mutation Assay

TOX CHEM No. 896E
TOX PROJECT No. 1185
Record No. 164326

FROM: John Doherty 7/28/86
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THRU: Edwin Budd, Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Budd 7/28/86

8/18/86

Background

The American Hoechst Company has submitted four mutagenicity studies in response to the data requirements indicated in the Registration Standard for this chemical. These studies were reviewed as below.

Toxicology Branch Comments

Two of the studies, chromosome aberration in cultured human lymphocytes and mouse lymphoma forward mutation assay, showed indications of a positive response. Toxicology Branch's position is that TPTH is presumptively positive in the chromosome aberration study. An additional study will have to be submitted to clarify the issue.

Although the mouse lymphoma forward mutation assay was borderline positive, TB does not consider an additional study necessary. TB recognizes that TPTH may have a low degree of mutagenic potential in this type (mouse lymphoma forward mutation) assay.

Studies Reviewed*

Study	Result	Comment
Human lymphocyte cytogenetic assay in vitro for chromosome damaging potential	Presumptively positive at dose levels at or near cytotoxic levels (0.5 ug/mL and 1.0 ug/mL).	Inconclusive**
Gene mutation in Schizosaccharomyces pombe	Negative up to and including cytotoxic levels.	Acceptable
Mouse Micronucleus	Negative at up to and including 77% of the LD_{50}, a dose level showing signs of TPTH toxicity.	Acceptable
Mouse Lymphoma forward mutation	Borderline positive at 250 and 300 ng/mL in the presence of S-9 mix. Negative in absence at up to and including cytotoxic levels (60-80 ng/mL).	Acceptable
Acute Oral LD_{50} - mice	About 180 mg/kg (probably higher in females).	Supplementary

*The conclusions of these studies were made based on the recommendations made by Dr. I. Mauer, TB geneticist.

**A second study will have to be conducted, submitted and reviewed and the issues of potential mutagenicity in this test system resolved.

2

Study to evaluate the chromosome damaging potential of HOE 029664 - substance technical by its effects on cultured human lymphocytes using an in vitro cytogenetics assay.

Microtest Research Ltd., Study No. HOF 2/HLC/KF17/HL1, August 13, 1985, EPA Accession No. 259354 Tab #1.

This type of mutagenicity study assesses the potential for chemicals to interfere with the process of mitosis or to cause chromosome aberrations.

The test materials used for this study were triphenyltin hydroxide (97.2% purity), methyl methanesulphonate (positive control for the study in absence of the liver S-9 activation system) and cyclophosphamide (positive control in the presence of the liver S-9 activation system). The liver S-9 activation system was prepared from the liver of rats (Wistar, males) dosed with Aroclor 1254 at 500 mg/kg in corn oil and sacrificed 5 days after treatment. The human lymphocytes were obtained from two donors, a male and a female, who were reported to be in good health. Their blood samples were cultured and phytohaemagglutinin added to stimulate the lymphocytes to divide. Other aspects of the study protocol were modeled after the OECD Test Guidelines No. 473. The test material was dissolved in DMSO prior to application to the cultures.

In the preliminary rangefinding study, test levels of 500, 200, 100, 50, 20, 10, 5, 2, and 1 ug/ml were tested in duplicate and each preparation had duplicate solvent controls. To one sample of each duplicate, the S-9 mix was added and 3 hours were allowed for the S-9 mix to react with any TPTH.

The results of the rangefinding study indicated the TPTH was toxic to the cells at all dose levels except for the lower dose levels (1-2 ug/ml) in the presence of the S-9 mix because the mitotic index was zero percent compared to 2.4 percent in the absence of S-9 mix and 1.6 percent in the presence of S-9 mix. On this basis, the dose levels of 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 ug/ml and 2, 1, 0.5, and 0.25 ug/ml were selected for the studies without the S-9 mix and with the S-9 mix, respectively. It is apparent that only one culture for each condition or concentration was run. The cells were scored by analyzing (when possible) 100 metaphases from each culture for chromosome aberrations. Cells with 44 or more chromosomes were acceptable for scoring. The method of classification of the aberrations was appended to the study. The mitotic index was determined by scoring metaphases in at least 500 cells. Statistical analysis employed the Chi-square formula.

Results

1. The positive controls for both the presence and absence of the S-9 mix responded as expected, producing an assortment of chromosome aberrations.

2. In the absence of the S-9 mix, TPTH also showed evidence of inducing chromosome aberrations which was statistically significant at both the 0.5 and 1.0 ug/mL dose levels. (P values of < 0.01 and < 0.001, respectively, for aberrations with gaps and < 0.05 and < 0.001 for aberrations without gaps.) Although there was some reduction in sample size (= less than 100 cells scored) due to the toxicity of TPTH at the 1.0 ug/mL dose level, the report maintains that this alone does not compromise the study.

3. In the presence of the S-9 mix, TPTH also showed statistically significant increases ($P < 0.001$) in aberrations with and without gaps, at 2.0 ug/mL (a level which was not cytotoxic).

The aberrations consisted mainly of chromatid deletions and exchanges and reached levels of "20X" the control. TB, however notes that in most cases there were no indications of chromosome aberrations in the controls but all doses of TPTH had some form of aberrations in the low dose groups. It is unclear where the "20X" multiple mentioned by the report comes from.

Conclusion

This study is considered INCONCLUSIVE. It presumptively demonstrates that TPTH, at and near dose levels which are cytotoxic produces chromosome aberrations in both the presence and absence of the S-9 activation system. A second study which demonstrates a clear NOEL will have to be conducted and submitted for review.

Study of the capacity of the test article HOE-029664 - substance technical grade (Code No. HOE 029664 OF 2D97 0004) - to induce gene mutation in Schizosaccharomyces pombe

Instituto di Ricerche Biomediche Antoine Marxer, Study No. M 889, August 20, 1985, EPA Accession No. 259345, Tab #2.

The purpose of this type of study is to detect forward mutations induced in five different genes of the yeast cell. Normally colonies are red, but mutated colonies are white. Overall, the red pigment occurs as a result of a mutation at the sixth of the ten genes that control the adenine biosynthetic pathway and if adenine in lacking in the growth medium, the colonies appear red. If a mutation occurs in one of the five genes preceding the sixth gene, the red pigment fails to accumulate and the colonies are white.

The test material used for this study was triphenyltin hydroxide (TPTH, 97.2% purity). It was dissolved in DMSO for use. Methylmethanesulfonate and dimethylnitrosamine were used as the positive control agents. The dose levels tested were 0.05, 0.1, 0.5, and 1.0 ug/mL for the test without S-9 metabolic activation and 1, 10, 50, and 100 ug/ml with the S-9 metabolic activation. These levels were selected based on preliminary rangefinding studies. Fourteen plates per determination were prepared for the suspensions in concentrated strength (50,000 cells/mL) and four plates per condition were used for diluted strength cell suspensions (about 5000 cells/ml). The ratio of mutated colonies to total surviving colonies gave the mutation frequency. The Chi-square method of statistics was used to evaluate differences in response to the test chemicals.

Results

The positive controls for both with and without the metabolic activation system gave the expected positive result.

In the absence of the metabolic system (S-9), TPTH was moderately to severely toxic to the cells at 0.5 ug/ml (45% survival) and 1.0 (20% survival). Lower doses (0.05 ug/ml, 88% survival, and 0.1 ug/ml, 69% survival) were minimally toxic. The frequency of mutations determined for all but the highest test dose (which could not be determined) were not statistically different from the control. The positive control (MMS) group had a mutation frequency of 36-fold higher than the DMSO control group.

In the presence of the S-9 (rat preparation) mix, only the high dose group (100 ug/mL, 64% survival) was moderately toxic to the cells. The mutation frequency was not statistically different from the DMSO control for any test group of TPTH. The positive control group was about 12 times the solvent control value.

The data were presented in summary form only. No raw data were presented with the study report. They are reported as being on file at the test laboratory.

Conclusion

This study is ACCEPTABLE. TPTH was not mutagenic under the conditions of this assay.

Mouse micronucleus test with TPTH technical

RCC (Switerzland), Study No. 049522, August 5, 1985.
EPA Accession No. 259345, Tab. No. 3.

The principle of the test is that in mammalian bone marrow cells acentric chromatids and chromosome fragments, induced by clastogenic agents or whole chromosomes induced by agents affecting the mitotic spindle, lag behind in the anaphase of cell division when the main nucleus is expelled from erythroblasts to form PCE's. Eventually they are included in the PCE and NCE cells where they are visible as micronuclei. Thus, agents which cause chromosome aberrations will result in increases in the frequency of micronuclei to be identified under the microscope.

The test article used for this study was triphenyltin hydroxide (TPTH, 97.2% pure). The positive controls were cyclophosphamide, 7,12-dimethylbenzanthracene, and ethyl-methanesulfonate. The test materials were prepared in either arachis oil or carboxymethylcellulose.

The test animals were mice (NMRI outbred, SPF) and they were of 5 to 7 weeks of age. They were dosed by gavage with TPTH at approximately 77 percent of the LD_{50} dose level or 140 mg/kg and at the lower levels of 70 and 35 mg/kg in carboxymethylcellulose (CMS). The positive control groups received 200 mg/kg of ethylmethanesulfonate (in CMS, 24-hour interval group only). The 48- and 72-hour positive control groups received 75 mg/kg of 7,12-dimethylbenzanthracene in arachis oil. The mice in the alternate positive control group received 50 mg/kg of cyclophosphamide in sodium chloride (0.9%) solution.

At 24, 48, and 72 hours after treatment six mice per sex per group were sacrificed but only five were examined for micronuclei, unless it was technically necessary to evaluate the last mouse. Following sacrifice, the femurs were removed and bone marrow samples taken and prepared for microscopic analysis using a stain method developed by Pappenheim. Two slides per mouse were prepared. The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was based on 100 erythrocytes scored per slide. Statistical analysis of the data were by the method of a one-rank test.

Results

The mice in the high dose group (140 mg/kg) showed signs of reaction typical of TPTH (sedation, ataxia, rough fur) which were most prominent. These signs were evident to a lesser degree in the lower dose test animals.

The positive control groups dosed with either ethylmethanesulfonate (3.7X negative control) or dimethylbenzanthracene (4.02X negative control) responsed as expected at 24 and 48 hours but there was no positive response (i.e., no increase in micronuclei) at 72 hours. The samples from the mice treated with cyclophosphamide were not assessed.

The test report maintains that TPTH was not positive in this study system at either 24, 48 or 72 hours postdosing. There were, however, some increases recognized which were not statistically significant, such as readings of 1.37 at both 24 and 48 hours after the 140 mg/kg dosed group vs. only 0.84 and 0.95 for the negative controls representing 1.63 and 1.44 times the negative control. This is considerably lower than the response to the positive control. This increase was not significant by the Steel-test (many-one rank test) applied to assess the significance of the differences of the number of scored micronuclei in 1000 polychromatic erythrocytes between treated control groups at 24, 48, and 72 hours.

Conclusion

This study is ACCEPTABLE. It demonstrates that under the conditions of this assay TPTH does not, at dose levels of about 70 percent of the LD_{50}, cause chromosome aberrations in the bone marrow. The potential for TPTH to be clastogenic or cause chromosome aberrations is not defined by this study because there was no assurance or determination that TPTH actually reached the bone marrow to be able to cause such an affect.

Mutagenicity evaluation of HOE 029664 - Substance technical (Code: HOE 029664 OF 2D97 0004) in the L5178Y $TK^{+}/^{-}$ mouse lymphoma forward muation assay

Litton Bionetics (the Netherlands), Study No. E-9406, August 1985, EPA Accession No. 259345, Tab 4.

In this assay forward mutations are assessed at the thymidime kinase locus enabling mutant (TK^{-}/TK^{-}) mouse lymphoma cells to grow in the presence of 5-trifluorothymidine, which is lethal to normal cells (TK^{+}).

The test materials used in this study were triphenyltin hydroxide (TPTH, 97.2% pure) and the positive controls ethylmethanesulfonate) (EMS) for nonactivation system and methylcholanthrene (MCA) an indirect mutagen requiring activation. The test material was dissolved in DMSO. Preliminary rangefinding studies revealed that TPTH was toxic to the indicator cells (mouse lymphoma cell line (L 64 784, $TK^{+}/^{-}$) derived from the L5178Y line. The dose levels tested were 10, 15, 23, 30, 45, 60, 80, 100, 125, and 150 ng/ml of TPTH for the assay in the absence of metabolic activation. The dose levels tested were 40, 70, 100, 150, 200, 250, 300, 400, 500, and 600 ng/ml for the assay in the presence of the S-9 mixture. Assays were run in duplicate, except for the control which was run in quadriplicate. The positive control EMS was tested at 0.25 and 0.40 uL/ml and the positive control MCA was tested at 2.5 and 4.0 ug/ml. The liver S-9 mix was prepared from rats pretreated with Aroclor 1254.

Results

The nonactivation system. The positive control (EMS) responded as expected, resulting in a moderate (40-50%) depression of colony growth and a 8- to 9-fold increase in total colony mutants. For example, the negative control had 194 total mutant colonies and the positive control had an average of 1703 total mutant colonies.

The severe toxicity of TPTH allowed only the cells treated at 80 ng/ml and below to be cloned for assessment. No evidence of an increase in total mutant colonies or mutant frequency was evident in this study.

The S-9 liver activation system derived from rat liver. The positive control (MCA, 208 total mutant colonies) resulted in the expected positive response and a 7- to 8-fold increase (to average of 1325) in total mutant colonies.

Only the cells dosed with 300 ng/ml and below were cloned and assessed for mutant effects. The dose levels of 250 and 300 ng/ml showed evidence of a dose-related positive response 1.42 and 2.09 times the background and solvent control, respectively, in total mutant colonies and 1.57 and 1.81 times the background and solvent control for the mutant frequency. The study report concludes that because the mutation frequencies at the two highest concentrations that could be cloned "did not come close" to a value that is twice the minimum (for the testing laboratory) criterion for mutagenesis, these increases were of a borderline effect (i.e., "weak" mutagen in this system).

Conclusion

This study is ACCEPTABLE. The study demonstrates that TPTH may have a potential (low degree) to induce forward mutations in the mouse lymphoma assay at concentrations that are near levels which are cytotoxic (at 250 and 300 ng/ml) in the presence of the S-9 mixture. A second study, clarifying the presumptive positive response noted in this study, is not considered necessary because other forward mutation type mutagenicity studies such as the Ames test (refer to the Registration Standard for TPTH for review) and other studies (this review) were not positive. Moreover, the high cellular toxicity of TPTH confounds the mutagenicity testing in vitro especially at levels which are cytotoxic.

Acute Oral Toxicity LD_{50} Study in Mice
RCC Project No. 050038, August 1985. EPA Accession No. 259345, Tab 3 (as Appendix F).

This study was conducted to estimate the acute oral LD_{50} of triphenyltin hydroxide (TPTH, 97.2% pure) in mice to assist in selection of the dose levels for the mouse micronucleus test.

Three groups of three male and three female mice were dosed with either 80, 150, or 300 mg/kg of TPTH and observed for mortality and reactions.

None of the mice in the low-dose group died, two of the males but none of the females in the mid-dose group died on days 3 and 7. All of the mice in the high-dose group died on days 3 to 8.

The symptoms reported were sedation (up to day 11), dyspnea and emaciation (up to day 11). Ataxia was noted in the high dose group. No symptoms were reported in the low-dose group.

No necropsy changes were noted in survivors. The lungs and intestines were reddened in the mice that died.

Conclusion

This study is SUPPLEMENTARY. An approximate LD_{50} of 180 mg/kg is established. The data provide useful information regarding to time of onset and duration of the symptoms following ingestion of TPTH.

REVIEWER

005617

JUL 30 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No. 8340-17: Triphenyltin Hydroxide - Review of Mutagenicity Studies: Gene Conversion in S. cerevisiae D4 and Unscheduled DNA Synthesis in Rat Primary Hepatocytes

TOX CHEM NO. 896E
TOX PROJECT No. 1266
Record No. 166079

FROM: John Doherty 7/28/86
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TO: Henry Jacoby, PM 21
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Registration Division (TS-767C)

THRU: Edwin Budd, Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Budd 7/28/86

Background

The American Hoescht Corporation has submitted two mutagenicity studies with triphenyltin hydroxide (TPTH) in response to the data requirements as indicated in the Registration Standard for this chemical. These studies were reviewed as below.

Studies Reviewed

Study	Results	Comments
Gene Conversion in S. cerevisiae D4	Negative up to and including 5 ug/mL nonactivation system and 15 ug/mL with the S-9 activation system.	ACCEPTABLE

1

Study	Results	Comments
Unscheduled DNA synthesis in rat primary hepatocytes.	Negative at up to and including dose levels that are cytotoxic (i.e., 0.5 ug/ml).	ACCEPTABLE

HOE 029664 - Substance Technical Grade:
Gene Conversion Test in S. cerevisiae D4

Instituo di Ricerche Biomediche "Antoine Marxer" (Italy) Study No. M890, October 24, 1985, EPA Accession No. 260962.

In this study type, mutagenic activity is evaluated as the capacity of the test substance to cause an increase in gene convertant frequency as revealed by growth in selective media or media deficient in adenine or tryptophan, because the susceptible genes are involved in the biosynthesis of these chemicals.

The test materials used for this study were triphenyltin hydroxide (TPTH, 97.2% pure) and the positive control substances methylmethane-sulfonate (MMS) and cyclophosphamide (CP). The TPTH was dissolved in DMSO for testing. The test dose levels used were selected based on preliminary rangefinding studies and were 0, 0.1, 1, 3, and 5 ug/mL for the nonactivation system and 1, 5, 10, and 15 ug/mL for the activation system. Four plates per condition were prepared. The test protocol was modeled after the EEC Guidelines (Annex V, 6th Amendment). The test organism, Saccharomyces cerevisiae D4 was originally supplied by the "Laboratorie di Mutagenesi e Differenziamonte" in Pisa, Italy. The S-9 mix was prepared from rats pretreated with 500 mg/kg of Aroclor 1254. Statistical evaluations were made with the Chi-square method.

Results

The positive controls responded as expected, giving convertant frequencies of 13 to 14 for the trp 5 gene and 19 to 20 for the Ade 2 gene in the presence of metabolic activation and 15 and 14 to 15 for these gene locuses when metabolic activation was absent.

TPTH did not show evidence of a positive mutagenic response in any condition. At the higher dose levels TPTH was clearly cytotoxic to the yeast cells.

Conclusion

This study is ACCEPTABLE. TPTH was not demonstrated to induce mutations in this study at dose levels up to and including cytotoxic levels.

Evaluation of Fentinhydroxide, Substance Technical Grade (Code: HOE 029664 2D97 0004) In the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

Litton Bionetics, Inc., Study or Project No. 20991, October 1985, EPA Accession No. 260962.

The principle of this study type is that rat liver hepatocytes will not take up 3H-thymidine into their DNA at significant levels unless an agent induces DNA damage and the cells respond by making more DNA to repair the damaged nuclear material.

The test materials used were triphenyltin hydroxide (TPTH, 97.2% pure) and 2-acetyl aminofluorene (2-AAF) used as a positive control. TPTH was tested at 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 ug/mL and the test material was incorporated into the media with DMSO. The net nuclear grain count of radioactivity was determined for 50 randomly selected cells on each coverslip for each assay replication (3). Unscheduled DNA synthesis was evaluated after 18 to 19 hours of incubation. The study report included an extensive discussion of assay acceptance criteria.

Results

The positive control gave the expected positive response and was 22- to 23-fold higher than the control for the UDS grains/nucleus. The test chemical TPTH at dose levels up to 0.5 ug/mL (higher doses were lethal to the cells) did not result in increases in UDS grains/nuclei or show other signs of a positive mutagenic response.

Conclusion

This study is ACCEPTABLE and demonstrates that TPTH at dose levels up to and including dose levels that are cytotoxic did not induce unscheduled DNA damage.

[Note: The raw data were not presented but are reported as being on file at Litton Bionetics.]

REVIEWER

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

006589

FEB 11 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No.: 8340-17 - Triphenyltin Hydroxide. Review of a mutagenicity study (Cytogenic test in bone marrow of Chinese Hamster) and overview of mutagenicity studies prepared by Dr. Kerry Dearfield.

TOX CHEM No.: 896E
TOX PROJECT No.: 8-0178
Record No.: 205884

FROM: John Doherty John Doherty 2/10/88
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Hazard Evaluation Division (TS-769)

TO: Lois Rossi
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Registration Division (TS-767)

THRU: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769) Budd 2/11/88

The American Hoechst Corporation has submitted a mutagenicity study assessing for the possible affects of triphenyltin hydroxide (TPTH) on chromosomes in the bone marrow of Chinese Hamsters following oral administration (gavage). This study was submitted because a previous study to assess for chromosome aberrations was found to be inconclusive by Toxicology Branch (TB, refer to review by J. Doherty dated August 18, 1987). TB has reviewed the current submission and has found the study to be ACCEPTABLE. Refer to review attached.

Dr. Kerry Dearfield of TB was requested to prepare an overview of the available mutagenicity studies with TPTH and to determine if the available data base was sufficient or if additional mutagenicity studies are required. Dr. Dearfield's report is attached. Based on Dr. Dearfield's overview, the following is concluded.

1. The data base satisfies the requirements for mutagenicity testing.

2. TB considers that the available data base does not indicate that TPTH presents a mutagenicity concern such that further action based on mutagenicity is necessary at this time.

3. According to Dr. Dearfield the human lymphocyte cytogenetic assay for chromosome damaging potential (Microtest Research #HOF 2/HLC/RF17/HL1, dated August 13, 1985, refer to review by J. Doherty dated August 18, 1986) should be classified as ACCEPTABLE and that the study demonstrates that TPTH has intrinsic clastogenic activity in human lymphocytes. Based on this recommendation, TB upgrades the study from INCONCLUSIVE to ACCEPTABLE.

4. No additional mutagenicity testing is required.

   NOTE: Dr. Dearfield mentioned the possibility that a test using human lymphoblastoid cells (TK6 cell line) may help elucidate if human cells are indeed more sensitive than rodent cells to the effects of TPTH (see page 5). After some discussion with Dr. Dearfield it was decided that it was <u>not</u> necessary to request a study of this type at this time because the bulk of the available TPTH mutagenicity data do not indicate an overt mutagenicity concern. If a stronger basis for a mutagenicity concern with TPTH develops in the future, then it may be appropriate to request this type of study concerning testing for effects related to differential sensitivity to human cells versus rodent cells.

DOCUMENTS SUBMITTED

DOCUMENT	Results	Conclusion
Cytogenetic test in chinese hamster bone marrow cells in vivo. Pharma Research Toxicol and Path. #86.1104 April 29, 1987.	Not considered positive at dose levels up to and including 80 mg/kg (HDT). Levels tested: 0, 20, 50 and 80 mg/kg.	ACCEPTABLE
Triphenyltin Hydroxide Evaluation of the Mutagenic Potential. Hoechst-Roussel Agri-Vet Co. (Authors: E.L. Carmines and J.S. O'Grodnick). October 7, 1987.	Referred to Dr. K. Dearfield for review.	No DER prepared.

Reviewed by: J. Doherty 2/10/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: K. Dearfield 2/10/88
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DATA EVALUATION REPORT

STUDY TYPE: 84-2 Genotoxicity Category Cytogenetic test in Chinese hamster bone marrow cells.

TOX. CHEM. NO.: 896E

ACCESSION NUMBER: 403711-02

MRID NO.: Not provided

TEST MATERIAL: Triphenyltin hydroxide (96.2%, batch #14118; C 06155117) CAS Number 76-87-9.

SYNONYMS: TPTH

STUDY NUMBER(S): 86.1104

SPONSOR: American Hoechst Corporation, Somerville, New Jersey

TESTING FACILITY: Pharma Research Toxicology and Pathology
Hoechst Aktiengesellschaft
Frankfurt, Federal Republic of Germany

TITLE OF REPORT: Evaluation of HOE 029664 OF ZD 0004 in the In Vivo cytogenetic test in bone marrow cells of the Chinese Hamster - Chromosome analysis.

AUTHOR(S): Dr. Mueller

REPORT ISSUED: April 29, 1987

CONCLUSIONS:

TPTH was not demonstrated to be genotoxic at dose levels of 20, 50 and 80 mg/kg. The highest dose level was considered to be marginally within the maximum tolerated dose level. There was partial inhibition of the mitotic index at 12 and 24 hours after treatment with 80 mg/kg thus giving some indications of cytotoxicity.

Classification: ACCEPTABLE

Special Review Criteria (40 CFR 154.7): N/A.

QUALITY ASSURANCE STATEMENT: A statement signed by the Quality Assurance unit official (Ap. Harston) indicating that three inspections were made was provided.

The purpose of this study was to assess for potential genotoxicity in vivo in the bone marrow cells following oral administration of triphenyltin hydroxide (TPTH) to Chinese hamsters. This method, assuming that the test material actually reaches the bone marrow, enables various types of chromosomal aberrations to be assessed.

1. Preliminary dose range finding study.

Groups of three male and three female Chinese hamsters (age and weight not indicated but assumed to be 10-14 weeks old as were the animals for the main study) were dosed with either 70, 80 or 90 mg/kg and observed for reactions.

At 90 mg/kg, 1 of the females but none of the males died. The symptoms reported were reduced spontaneous activity, narrowed palpebral fissures, abdominal/lateral position and impaired general condition. The time of onset and duration of the symptoms were not reported.

At 80 mg/kg some of the same symptoms were reported but no hamsters died. No symptoms were reported in the group receiving 70 mg/kg.

On this basis, the dose level of 80 mg/kg was determined by the testing laboratory to be the maximum tolerated dose for use in the definitive study. TB notes the symptoms seen at 80 mg/kg were vague; it would have been desireable to test higher doses in the cytogenetic study.

2. Cytogenetic study.

Thirteen groups of 5 male and 5 female Chinese hamsters (10-14 weeks of age) were dosed with either vehicle (starch mucilage), 20, 50 or 80 mg/kg of TPTH such that one group from each dose level was sacrificed at 12, 24 or 48 hours after treatment. A positive control group (100 mg/kg of Endoxan®, cyclophosphamide) was included and sacrificed 24 hours after treatment. Two hours before sacrifice (by carbon dioxide asphyixation) the hamsters were dosed with 3.3 mg/kg of Colcemid® intraperitoneally.

Following sacrifice, the bone marrow was collected and prepared for microscopy. The preparation procedure consisted of hypotonic treatment in 0.075 M KNO_3 (TB notes that KCl is usually used) and fixation in methanol/glacial acetic acid. Staining consisted of 10 min in 0.2% orcein solution, rinsing with water, acetone and acetone/xylene and embedding in Entellan® or Enkitt®.

Evaluation consisted of microscopically examining 50 metaphases from each hamster. "The set of chromosomes was examined for completeness and various chromosomal aberrations were assessed." The metaphases were examined in particular for the following aberrations: gaps, breaks, fragments, minutes, deletions, exchanges including interchanges, rings, polyploidy and for multiple aberrations (five or more aberrations).

The criteria for determining a positive response to a test material were not provided in the study report.

Results.

1. Reactions to treatment. Only a summary statement was provided and the symptoms reported are vague. For example, the report states that the first signs of intoxication appeared after 24 hours in 3 animals for the group treated with 80 mg/kg of TPTH. These symptoms consisted of "impaired general condition". Forty eight hours after treatment some of the hamsters in the 50 and 80 mg/kg dose groups had blood stained nasal discharge, blood stained lacrimation, narrowed palpebral fissures and impaired general condition.

In the cytogenetic assay there was partial inhibition of the mitotic index at 12 and 24 hours after treatment with 80 mg/kg, thus giving some indications of cytotoxicity.

2. Genotoxicity.

Table 1 (attached, copied from the study report) presents the results of the analysis of the metaphases for chromosmal aberrations. The testing laboratory's assertion is that administration of TPTH "did not lead to a substantial increase in chromosomal aberrations". Thus, TPTH is not mutagenic in the in vivo cytogenetic test in bone marrow cells of the Chinese hamster.

The study report maintained that the positive control group produced the expected positive response. For example, there were 6.8 and 8.8% aberration frequencies "inclusive gaps" for the males and females respectively vs either 0 or 0.4% for the controls. Similarly there were 6.8 and 8.4% aberration frequencies "exclusive gaps" for the males and females respectively vs 0% for the controls.

TB notes that on inspection of the data at 24 hours for "inclusive gaps" a suggestion of a positive response is apparent. The suggestion is less obvious for the more important criteria of "exclusive gaps". This suggestion of a possible response is only evident at 24 hours. TB does not consider the suggestion of a possible positive response by these data sufficiently strong enough to warrant a conclusion that TPTH is positive in this study or to justify a request for a second study.

CONCLUSION. This study is considered to be ACCEPTABLE and provides a demonstration that TPTH did not produce signs of genotoxicity under the conditions of the assay.

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triphenyltin Hydroxide - Overview of Mutagenicity Studies

FROM: Kerry L. Dearfield, Ph.D.
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2.4.88

TO: John Doherty, Ph.D.
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THRU: Reto Engler, Ph.D.
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2/9/88

Triphenyltin Hydroxide [76-87-9] TOX CHEM NO. 896E

This memo is in response to your request to perform an overview of the available information concerning the potential mutagenicity of triphenyltin hydroxide. Most of the information is contained in the submitted studies to OPP in support of the data requirements for this chemical. The majority of this information was reviewed from the Data Evaluation Records (DERs) you provided. There are a total of nine (9) submitted studies to examine. Additional information, where available, is found in the published literature.

The nine submitted studies examining the potential mutagenicity of triphenyltin hydroxide are (with overall result and classification):

Gene Mutation Tests

Salmonella assay - negative, acceptable
S. pombe assay - negative, acceptable
Mouse lymphoma assay - weak positive, acceptable

Structural Chromosome Aberration Tests

Mouse micronucleus - negative, acceptable
Human lymphocyte cytogenetics - positive, acceptable
Chinese hamster in vivo cytogenetics - negative, acceptable
Rat dominant lethal assay - negative, acceptable

Tests for Other Genotoxic Effects

Gene conversion S. cerevisiae - negative, acceptable
Unscheduled DNA synthesis/primary rat hepatocytes - negative, acceptable

Specific information and data can be found in the DERs for each of these studies. I will refer to particular information in the ensuing discussion when necessary. The submitting company, Hoechst-Roussel Agri-Vet Company, has submitted their evaluation of the mutagenic potential of triphenyltin hydroxide (EPA MRID No. 40711-01). I will also address this evaluation where appropriate.

RESULTS

Gene Mutation Assays

Triphenyltin hydroxide (TPTH) was tested in the Salmonella assay ± activation in strains TA98, TA100, TA1535, TA1537 and TA1538. TPTH appeared quite toxic and the top concentration assayed was 5 ug/plate. No increased revertant frequencies were noted. TPTH was also tested in E. coli WP2 uvrA ± activation, but was not apparently as toxic to these bacteria for the highest concentration tested was 5000 ug/plate (saw some precipitate). Again, no activity was noted. Data from the published literature provide additional support to these submitted negative results (Moriya et al., 1983; Dunkel et al., 1985). For example, Dunkel et al. tested TPTH in the same Salmonella and E. coli strains listed above and found similar toxicity for Salmonella and no increased mutagenic activity in either bacteria.

TPTH was examined in the Schizosaccharomyces pombe assay for mutations in genes involved in the adenine biosynthetic pathway. At concentrations up to 1 ug/ml (20% relative survival) without activation and up to 100 ug/ml (64% relative survival) with activation, no increased mutant frequencies were observed.

There appeared to be mutagenic activity induced by TPTH in the mouse lymphoma gene mutation assay with activation. Concentrations up to 300 ng/ml were tested (higher concentrations were apparently toxic) with percent relative growth of 83.6% to 10.7% induced (the DER did not indicate which concentrations were associated with which survivals). A dose-dependent increase in total mutant colonies and mutant frequency was observed. This activity appears to be weakly positive as the frequencies were above the testing laboratory's minimum criteria for a positive response (125.3×10^{-6}). However, the increases were 1.57 and 1.81 times the background and solvent controls for 250 and 300

ng/ml, respectively; these are very slight increases. At concentrations up to 80 ng/ml and 20.9% survival, no activity was noted under non-activated conditions.

The National Toxicology Program (NTP) has also tested TPTH in the mouse lymphoma assay, but only without activation (W. Caspary, personal communication). They found similar toxicities and tested TPTH to a top concentration of 140 ng/ml. In disagreement with the submitted study, the NTP found TPTH active in the mouse lymphoma assay without activation. For example, at 100 ng/ml and 16.4% relative total growth, a 2.3 times background mutant frequency was found (156 X 10^{-6} vs. 67 X 10^{-6} background). The mutation induction appeared to plateau with higher concentrations and relative total growths dropped below 10%. A repeat found the same results. Based on both of these studies, it appears that TPTH is active in the mouse lymphoma assay at the thymidine kinase locus, albeit weakly so.

Structural Chromosome Aberrations

Human lymphocyte cultures from healthy male and female donors were exposed to TPTH for 3 hours ± activation. Cultures were incubated for a total of 73 hours following establishment (about 27-28 hours after start of treatment) before being harvested for assay. Without activation, a dose related increase in all aberrations, frequency of aberrations/100 cells and percent of cells with aberrations, all excluding gaps, was induced by TPTH. Chromosome and chromatid deletions were primarily observed. Less than 100 metaphases were counted at the top concentration of 1 ug/ml due to reduced mitotic index. At the next lower concentration, 0.5 ug/ml, there was a slight reduction of the mitotic index. At both of these concentrations there was a significant increase in aberration frequency with levels of aberrations exceeding the positive control (50 ug/ml MMS) at 1 ug/ml. A positive response was also observed under activated conditions. At the top concentration of 2 ug/ml (about 40% reduction of mitotic index), large increases in induced aberrations excluding gaps were seen. Slight increases were seen at 0.5 and 1 ug/ml, but these were not statistically significant. Aberrations observed were primarily chromosome and chromatid deletions, chromatid exchanges and some other aberration types (not detailed but including endoreduplication, hyperdiploidy, polyploidy and/or pulverized chromosomes). Overall, this study demonstrates that TPTH has intrinsic clastogenic activity. The original DER states that this study was inconclusive and that a second study is needed to demonstrate a clear NOEL. This is not a criteria for rejecting a positive mutagenicity study and this study needs to be reclassified as ACCEPTABLE.

A mouse micronucleus assay was performed with TPTH at doses up to 140 mg/kg (77% of LD50). Five animals/sex/dose were given single doses p.o. and bone marrow was obtained at 24, 48 and 72 hours post-treatment for analysis. Clinical signs (sedation, ataxia, rough fur) were seen, most prominently at 140 mg/kg. No

dose related or time related decreases in the ratio of polychromatic erythrocytes to normochromatic erythrocytes were observed, indicating no cytotoxicity by this parameter. Very marginal, but not statistically significant increases in micronucleus induction were seen. Overall, TPTH did not appear to be positive under the conditions tested in this assay.

Another in vivo cytogenetics assay was performed with TPTH in Chinese hamster bone marrow. Five animals/sex/dose were given single doses p.o. and bone marrow was obtained at 12, 24 and 48 hours post-treatment for aberration analysis. The top dose of 80 mg/kg produced some clinical signs (not detailed) and there was some depression of the mitotic index at the 12 and 24 hour sample times. There were no apparent significant increases in aberration frequency at any assayed dose (there was a slight marginal increase in the 24 hour sampled females).

A rat dominant lethal study with doses of TPTH up to 150 mg/kg assayed was submitted. Ten males/group were dosed daily by gavage for 5 days and then sequentially mated to 2 females/week for 10 weeks. Eight of the high dose males died; some of the rats in the next lower dose group, 38 mg/kg, had loose hair, but no other signs reported. There did not appear to be a dominant lethal effect up to a dose of 38 mg/kg. There were possible dominant lethal effects at the 150 mg/kg dose, but may have been obscured by the high mortality and the reported poor overall health in this test group (see DER for details). A mouse dominant lethal study using triphenyltin acetate was reported in the literature (Epstein et al., 1972). This report states there was no dominant lethal effect up to doses that caused mortality; however, this report is not completely adequate as individual data were not presented to make an assessment.

Other Genotoxic Effects

Primary rat hepatocytes were exposed to TPTH for 18 - 19 hours at concentrations up to 2 ug/ml and then were examined for unscheduled DNA synthesis (UDS). At concentrations up to 0.5 ug/ml no increased net nuclear grains over control were found. Higher concentrations were lethal to the cultured hepatocytes. The DER states that raw data were not submitted.

TPTH was tested in the Saccharomyces cerevisiae D4 assay for gene conversion. At concentrations up to 5 ug/ml without activation and up to 15 ug/ml with activation, negative responses were seen at the trp5 and ade2 genes. Higher concentrations were cytotoxic to the yeast cells.

DISCUSSION

The submitted studies present a database that satisfies the required data requirements for mutagenicity testing. The weight-of-the-evidence suggests that there is little support for a mutagenicity concern. The major positive finding is with the cultured human lymphocytes assay. However, the bulk of the in

11

vivo data suggest that there may not be a large concern when test animals are exposed to TPTH. The lack of reported germ cell interactions or effects do not suggest a heritable concern. This is in general agreement with the overall assessment submitted by Hoechst.

A larger concern may be its apparent acute toxicity. TPTH is extremely cytotoxic in vitro and appears to cause lethality at doses below 200 mg/kg. The cytotoxicity of organotin compounds in mammalian target organs such as the central nervous and immune systems has been recently reviewed (Snoeij et al., 1987). This aspect should perhaps be pursued.

Another aspect that surfaces in these studies is the apparent differential sensitivity of human cells as compared to rodent cells. The response in the cultured human lymphocytes are significant and cannot be demonstrated to be a false positive in this assay at this time. This is in disagreement with the Hoechst evaluation that states this is a false positive in this study. It may be worth pursuing this potential differential sensitivity with additional studies utilizing human cells. For instance, a gene mutation assay using human lymphoblastoid cells (TK6 cell line) may help elucidate if human cells do indeed respond to TPTH. Based on the mouse lymphoma results above at the thymidine kinase locus, possible mutation at this locus in the TK6 cells may be worth examining.

REFERENCES

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cc: E. Budd
Section Chief