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

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SEP 2 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No.: 8340-17 - Triphenyltin hydroxide. Review of registrants summary paper on immunotoxicity and an immunotoxicity study testing host resistance in rats to Trichinella spiralis.

TOX CHEM No.: 896E
TOX PROJECT No.: 7-0765
Record No.: 197059

FROM: John Doherty 8/18/87
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Lois Rossi
Product Mananger #21
Registration Division (TS-767)

THRU: Edwin Budd 8/18/87
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769)

Background

Triphenyltin hydroxide (TPTH) has been implicated as being potentially immunotoxic (refer to the Toxicology Branch Chapter for the Registration Standard). The registrants were previously asked to summarize the studies available which assess for potential immunotoxicity and in particular to respond to certain comments made by Toxicology Branch (TB) regarding the NOEL set for the special immunotoxicity study conducted by the Quintox Laboratory. The registrant, the American Hoechst Co. has recruited the expertise of Dr. W. Seinen, a reputed expert on immunotoxicity to assist with this evaluation. Dr. Seinen's report has been submitted to the Agency together with a new study designed to assess for potential immunosuppression.

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Toxicology Branch Comments

1. The study designed to assess for potential immunosuppressive effects of TPTH using Trichinella spiralis larvae was determined to be UNACCEPTABLE. The assay used has not been sufficiently validated such that the optimum conditions to assess for a test chemical effect are known. The compound used as the positive control gave at best a weak positive response.

2. The summary of potential immunotoxicity of TPTH prepared by Drs. Seinen and Penninks was reviewed by Dr. Roy Sjoblad of TB (refer to memo attached). Based on review of this summary as well as other factors, Dr. Sjoblad has concluded that "A NOEL for effects of TPTH on cellular components of the immune system has not been provided..". Thus, the problems and issues related to immnotoxicity testing as indicated in the Toxicology Branch of the Registration Standard for TPTH have not been resolved.

3. TB's current position (August, 1987) is that additional data are needed to establish a NOEL for potential immunotoxicity.

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STUDIES REVIEWED

Study Type	Results	Classification
Immunotoxicity-rats (Host resistance to Trichinella spiralis) Authors: K.-H. Diehl and K.-H. Leist, Hoechst Aktiengesellschaft No. 85.0986, March 14, 1986.	Study type is not considered sufficiently validated to be useful at this time.	UNACCEPTABLE
Immunotoxicity - Summary entitled: Immunotoxicity of Triphenyltin Compounds. Authors: Prof. Dr. W. Seinen and Dr. A. H. Penninks. Date April 1, 1987 EPA Acc. No.: 402003-02. [Reviewed by R. Sjoblad]	See review attached for TB comments.	N/A

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

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DATA EVALUATION REPORT

STUDY TYPE: Immunotoxicity (Special) TOX CHEM NO.: 896E

ACCESSION No: 402003-01 MRID NO.:

TEST MATERIAL: Triphenyltin hydroxide, Test [illegible]%

SYNONYMS: TPTH

STUDY NUMBER(S): 86.0772

SPONSOR: American Hoechst Corporation

TESTING FACILITY: Pharma Research Toxicology and Pathology (Frankfurt, Germany)

TITLE OF REPORT: Testing of host resistance in the female Wistar rat (Immunotoxicological screening with Trichinella spiralis).

AUTHOR(S): Dr. K.-H. Diehl and Dr. K.-H. Leist

REPORT ISSUED: March 14, 1986

CONCLUSIONS:

The assay used is not considered by TB to be sufficiently validated. Parameters such as dosing duration time, time of assay of the tongues relative to dosing with the test material and the infectious agent, the expected response to the positive control as well as others are not considered either optimized or otherwise sufficiently documented.

There were also problems in the conduct of the study itself. Only a single dose level was used and no systemic effects of TPTH resulted.

Classification: UNACCEPTABLE

Specical Review Criteria (40 CFR 154.7) - N/A

REVIEW

The purpose of this study was:

-"to test the feasibility of a test method for determining host resistance.

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- to determine the immunosuppressive effect of triphenyltin hydroxide (TPTH).....on host resistance to rats".

This assay type is a new and unvalidated method to assess for possible immunotoxicity and was introduced in October of 1985 by Dr. J. Vos of the National Institute of Public Health and Environmental Medicine in Bilthoven (Neitherlands).

Procedure.

The study to assess for possible immunotoxic potential of TPTH consisted of three groups of 10 female Wistar rats each.

-Group I: dosed by gavage with 2.5 mg/kg/day of TPTH in sesame oil for 10 successive days.

-Group II: dosed intraperitoneally twice and at 5 day intervals with 50 mg/kg of cyclophosphamide in water. This group was the positive control.

-Group III: dosed by gavage with sesame oil on 10 successive days. This group was the vehicle control group.

After dosing as above the rats were infected with Trichinella spiralis larvae. The mechanism of dosing was not described for the main study but a reference to oral dosing was made in the pilot study. The mechanism of dosing should be thoroughly described and it should be made clear how each animal was actually dosed with "580 infectious Trichinella larvae". It is unclear how such an exact count was administered and what prevented the rats from spitting out some of it. Half of the rats from each group were sacrificed on day 20 and the other half were sacrificed on day 50 following infection. After sacrifice (by carbon dioxide asphyxiation), the tongues were removed from each rat and prepared for analysis for Trichinella larvae.

Results.

There was no effect on body weight gain (the only parameter investigated for systemic effects of treatment) with either TPTH or cyclophosphamide.

Table c (attached, zeroxed from the study report) shows the summary of the test results for analysis of Trichinella larvae. A positive result would have been indicated by an increase in the number of Trichinella larvae after either 20 or 50 days or on both of these days. Table c shows that for the rats dosed with

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TPTH there were decreases in the Trichinella larvae counts compared with the solvent control. These results do not indicate an immunosuppressive effect of TPTH but suggest that TPTH may have been toxic to the larvae. [Although TPTH toxicity to the larvae is a possible explanation for the decrease in larvae count, it is only a possibility because the rats were infected with the larvae one day after the last dose of TPTH. A time when most of the TPTH would have been excreted. The positive control group dosed with cyclophosphamide had larvae counts higher than the vehicle control on both daya 20 and days 50 but on neither day did the count exceed two fold.

CONCLUSION

This study is UNACCEPTABLE. TB cannot accept this study as a demonstration that TPTH is not immunosuppressive.

The assay procedure of utilizing Trichinalla larvae to assess for immunosuppression does not have a validated protocol. Many parameters such as the time course for chemical treatment and dose levels or methods of administration as well as other critical parameters have not been optimized. Although the positive control cyclophosphamide treated groups showed some evidence of an immunosuppressive effect, it was considered by TB to be only s slight increase and not a two fold increase as would be expected for a positive control (without thorough documentation showing otherwise). This study type needs to be validated using several chemicals and appropriate historical control data for this assay presented before TB can utilize this study type for immunotoxicity evaluation.

There were also problems with the assay itself as conducted. These include that only a single dose level of TPTH was used and this level did not result in pharmacological responses in the rats. In most any study submitted to TB, the high dose, unless it is very high and meets criteria for a limit test, must show responses to the test chemical. Another problem is that the rat tongues were assessed for Trichinella larvae 20 and 50 days after the last dose of TPTH. In the absence of documentation that all immunosuppressive agents have their primary effect immediately following acute dosing (as was done with cyclophosphamide) or the multiple dosing (10 succsssive doses of TPTH), there is no basis for selecting the assay times for Trichinella larvae counts at 20 and 50 days. At these time intervals, TPTH, would be expected to have been eliminated from the body. Thus any immunosuppressive effect would be secondary to an earlier effect of the chemical on the immune system.

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Host resistance assays where death of the treated animals is the endpoint have been used previously to demonstrate an immunosuppressive effect of TPTH (Vos et al, Toxicology 29: 325-336, 1984). In this study rats dosed with 25 mg/kg showed less resistance to Listeria monocytogenes infection.

[Note: This review and the study itself have been read by Roy Sjoblad, Ph.D. and several of his comments have been incorporated.]

c) Summarised results of Trichinella larva counts

Group	Number of Trichinella larvae $\bar{x}$ after 20 days	% control	Number of Trichinella larvae $\bar{x}$ after 50 days	% control
I TPTH	10	91	23	74
II Cycloph.	16	145	47	152
III Control	11	100	31	100

Statistical examination revealed that only the Trichinella larva count 50 days after infection showed a significant increase as compared with the control.

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PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of summary report of Drs. W. Seinen and A. H. Penninks (University of Utrecht, Netherlands) on literature concerning the "Immunotoxicity of Triphenyltin Compounds" (Report date: April 1, 1987). Accession No. 40200[illegible]

TO: Edwin Budd
Head, Review Section II
Toxicology Branch

FROM: Roy D. Sjoblad, Ph.D.
Microbiologist
Scientific Mission Support Staff

R.D. Sjoblad 5/5/87

I. Background and conclusions of Seinen and Penninks:

American Hoechst Co. has submitted to the Agency a summary report on existing literature and laboratory studies that contain data relevant to the immunotoxicological potential of triphenyltin (TPT) compounds, and in particular, triphenyltin hydroxide. The summary report was authored by Prof. Dr. W. Seinen and Dr. A. H. Penninks from the University of Utrecht.

The report authors concluded from their review of existing literature and laboratory studies, that "...[L]ymphopenia and lymphocyte depletion of spleen and thymus are the most sensitive effect parameters of TPT-treatment." The authors also concluded that triphenyltin compounds display "...immunosuppressive potential, but only at dose-levels that significantly reduce the number of lymphocytes in peripheral blood and/or the weight of the lymphoid organs thymus and spleen." In addition, the authors felt that the data show that the effects of triphenyltin compounds on the lymphoid system "appear to be transient" and are "diminished" after long term (e.g., 13-weeks) treatment.

II. Toxicology Branch reviewer conclusions on Seinen and Penninks report:

This reviewer agrees with the conclusion of the Hoechst Co. summary that lymphopenia and lymphocyte depletion of spleen and thymus are sensitive effect parameters of triphenyltin hydroxide (TPTH) treatment, but does not agree that existing data are sufficient

to demonstrate that these effects are transient and become diminished with longer periods of dosing. Further, the data do not allow for a conclusion that risks due to immune system perturbations from exposure to TPTH are acceptable. In addition, the summary report does not satisfy the long-standing Toxicology Branch request that Hoechst Co. provide data which establish a NOEL for TPTH on certain cellular components of the immune system.

This reviewer will categorize and summarize the relevant information presented in the report, and then will address the information in these summarized categories for the purpose of supporting his conclusions.

III. Summary of specific relevant information in Seinen/Penninks report.

A. General information:

1. The report authors state that the toxicity of triphenyltin compounds is independent from the nature of the anionic group, and thus toxic effects reported for triphenyltin hydroxide (TPTH), triphenyltin acetate (TPTA), and triphenyltin chloride (TPTC; or for the purposes of this Memorandum, abbreviated TPhTC) can be considered as equivalent.

B. Reported NOELs and LELs: lymphopenia and lymphoid organ weights:

1. " In guinea pigs a significant lymphopenia was observed in both males and females fed TPTH from levels of 10 and 2.5 ppm, respectively."

2. The report authors conclude from their review of a Hoechst A.G. sponsored study ("Immunotoxicological study in male mice"; Quintox Inc., Study No. QU/THAN 104; May 1982) that "...decreased leucocyte counts were found at dose levels of 2.5, 5, and 20 mg/kg..." and, TPTH-treated mice that were immunized with sheep red blood cells, "...displayed a dose-related decrease in spleen weight as well as in the number of spleen cells from a level of 2.5 mg [TPTH]/kg body weight."

C. Reported NOELs and LELs: Immune function assays, immunoglobulins, and host resistance to infection:

1. In some guinea pigs, especially at the 50 ppm level, a mycotic infection was noted in the gastrointestinal tract and the mesenteric lymph nodes, which may be indicative of a decreased resistance against infection."

2. In general, and based on the limited number of studies thus far done, measured immune function parameters are affected by TPTH, "...but only at dose levels that already markedly reduced spleen weight and spleen cell numbers."

3. While earlier studies did not indicate any effects of TPTH on serum immunoglobulin (Ig) levels, decreased serum Ig levels

were observed in a recent 13-week TPTH feeding study with mice (RCC Report; January 20, 1986). Seinen and Penninks dismiss these statistically significant ($p < 0.01$) results (see June 9, 1986 Memorandum from R. Levy to J. Doherty) as "an isolated finding which can not be interpreted as an immunotoxic effect of TPTH..." because the method used (radial immunodiffusion assay) to measure the Ig levels "...is outdated because of lack of accuracy..." and no other immunotoxic effects were observed in the same study.

D. Evidence that short-term exposure to TPTH causes only transient interactions with the lymphoid system, which disappear after long-term exposure:

1. "In two weeks feeding study with male weanling Wistar rats Snoeij et al....described a dose-related reduction of spleen weights at dietary levels of 50 and 150 ppm TPTC...These spleen effects appeared to be transient, since feeding of 100 ppm TPTC for 4 weeks did not affect spleen weight."

2. No lymphoid organ abnormalities were observed in rats or mice fed with TPTH at 0, 4, 20, or 100 ppm TPTH for 13 weeks (Hoechst Report No. 637/81, November 1981; and, RCC Report, January 1986, respectively).

IV. Toxicology Branch reviewer critique and considerations of the Seinen/Penninks report:

A. Reported NOELs and LELs: lymphopenia and lymphoid organ weights:

1. None of the studies summarized in the report establish a NOEL for effects of TPTH on lymphopenia and lymphoid organ weights. The lowest effect level reported for these parameters was 2.5 ppm in the guinea pig, equivalent to approximately 0.1 mg/kg/day.

2. Seinen and Penninks concur with the Toxicology Branch observation that the lowest dose level of TPTH used (i.e., 2.5 mg/kg) in the Quintox, Inc. study (sponsored by Hoechst, Co.) affected spleen weight and spleen cellularity. Thus, the Toxicology Branch conclusion (see Memorandum from J. Doherty to H. Jacoby; July 15, 1986) that a NOEL for TPTH was not established in this study, is supported by Seinen and Penninks.

B. Reported NOELs and LELs: immune function assays, immunoglobulins, and host resistance to infection:

1. Evidence that TPTH may interfere with host resistance to infection by microrganisms was presented; An in-depth evaluation of effects of TPTH on host resistance to microbial and tumor cell challenge using the most sensitive and established animal model systems currently available may be required as components of a risk-determination study.

2. There are a sufficient number of studies that exist which show that TPTH can effect immune function parameters. Drs. Seinen and Penninks apparently equate only functional immune assay parameters with immune suppression, while excluding from this category //

signs of lymphocyte depletion in blood and lymphoid organs.

3. The Registrant has submitted data that show a statistically significant effect of TPTH on serum immunoglobulins. Irrespective of the limitations of the technique used, the reported adverse effect should be resolved. This reviewer suggests that the Registrant use a more sensitive and accurate technique to provide a full evaluation of effects of TPTH on serum immunoglobulin levels.

C. Evidence that short-term exposure to TPTH causes only transient interactions with the lymphoid system, which disappear after long-term exposure.

1. There are no studies available where the lymphoid system effects of short-term dosing with TPTH disappeared upon longer-term dosing of the same test animals.

2. In one study (H.G. Verschuuren, et al., 1970, Tox. Appl. Pharm., 16:400-410) female guinea pigs dosed with TPTA for 104 days showed decreases in "...the number of gamma-globulin-containing cells and the number of tetanus antitoxin-producing cells..." in the popliteal lymph nodes, and "...no recovery was observed after an additional 2 weeks without treatment."

3. Seinen and Penninks referred to the study of N.J. Snoeij, et al. (Tox. Appl. Pharm., 81:274-286) to support their suggestion that TPTH effects disappear as the dosing period lengthens. However, their comments are relevant only for TPTC - which is the abbreviation for tri-n-propyltin chloride used by Snoeij, and not triphenyltin chloride. The test animals were not dosed with 100 ppm for 4 weeks with triphenyltin chloride - abbreviated as TPhTC in the journal article.

V. Toxicology Branch reviewer discussion:

Sufficient information is available to allow for the conclusion that TPTH at low concentrations in the diet can affect lymphocyte levels in the blood and lymphoid organs, and lymphoid organ weights, and at higher dose levels can affect immune functions. Evidence provided by Hoechst has shown an effect of TPTH on serum immunoglobulin levels. A NOEL for effects of TPTH on cellular components of the immune system has not been provided by Hoechst. In addition, consequences of lymphopenia and lymphocyte depletion in lymphoid organs due to chronic exposure to low levels of TPTH are not known.