CASWELL FILE 2

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

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

PC 083601

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
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Lois Rossi
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THRU: Edwin Budd
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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.

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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.

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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
Scientific Mission Support Section, Tox. Branch (TS-769C)

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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.

REVIEW

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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®.

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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.

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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.
Geneticist
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Kerry L Dearfield 2.4.88

TO: John Doherty, Ph.D.
Toxicology Branch
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THRU: Reto Engler, Ph.D.
Chief
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Toxicology Branch
Hazard Evaluation Division (TS-769C)

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. 40[illegible]711-01). 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 $\pm$ 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

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

Dunkel V, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz H, Simmon V. 1985. Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in Salmonella typhimurium and Escherichia coli. Environ Mutagen 7 (Suppl 5): 1 - 248.

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Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat Res 116: 185 - 216.

Snoeij J, Penninks A, Seinen W. 1987. Biological activity of organotin compounds - an overview. Environ Res 44: 335 - 353.

cc: E. Budd
Section Chief

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R054729

Chemical:	**Fentin hydroxide**
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HED File Code	**13000 Tox Reviews**
Memo Date:	**02/11/88 12:00:00 AM**
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