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

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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
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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.

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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.

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 phyto-haemagglutinin 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 ethylmethanesulfonate. 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.