1-23-89

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

OK

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: California Department of Food and Agriculture - EPA Toxicology Review for lindane.

TOX CHEM No.: 527

FROM: John Doherty 1/23/89
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Budd 1/23/89

The California Department of Food and Agriculture Medical Toxicology Branch lists as Data Gaps for the insecticide lindane the following studies:

Reproduction, rat

Mutagenicity: Chromosome mutation (aberration)

Mutagenicity: DNA damage

[Refer to the document entitled "Summary of Toxicology Data LINDANE (gamma hexachlorocyclohexane) prepared December 11, 1986 and revised October 24, 1988 for SB 950-073, Tolerance #133. attached.]

The Toxicology Branch Chapter for the Registration Standard for Lindane indicated that there were studies available which were either CORE MINIMUM (the reproduction study) or ACCEPTABLE (the mutagenicity studies) and that there were no Data Gaps for these study types.

The remainder of this memo will address CDFA's concerns and TB-I's responses regarding the three study types listed above.

1. Rat reproduction study.

[Study identification: "Effect of Lindane on Reproductive Function of Multiple Generations in the Rat." Huntingdon Research Centre, Report #4239 71/445, 2/16/72.]

A. The following are the comments as made by the CDFA:

"Lindane (Batch No. 6801/403, ≥ 99.0%) tested at 0, 25, 50 and 100 ppm in the diet in a 3-generation study in Charles River CD rats; 10 male and 20 female rats/dose group for all generations; increase in liver weight in F3 pups at 100 ppm; NOEL = 50 ppm; NO ADVERSE EFFECT; INCOMPLETE; UNACCEPTABLE (dose levels not high enough, insufficient histopathology and necropsy, unknown clinical observation intervals, and too few males). Schreider 4/22/85, Davis 9/21/88."

B. TB-I response:

i. TB-I considers this study to be CORE MINIMUM. The conduct and reporting of the study was sufficiently detailed to conclude that at the dose levels tested lindane did not affect the reproductive (either male or female) performance of rats under the conditions of the study. The study may be used to satisfy the data requirement for a multi-generation reproduction study with lindane.

ii. "dose levels not high enough". The study established a NOEL of 50 ppm and a LEL of 100 ppm for systemic effects (liver weight changes). A NOEL of 100 ppm (the highest dose level tested) was also assigned for reproductive effects. Considering other available rat studies on lindane, the lowest NOEL for systemic effects currently used by TB-1 is 4 ppm based on kidney effects noted in a subchronic (90-day) rat feeding study. There is also a rat chronic feeding study currently being conducted that will further assess for the systemic effects of lindane at dose levels in the range of 4 ppm and above. TB-I does not consider it necessary to repeat the reproduction study at higher levels than 100 ppm to assess for toxicity when the NOEL for systemic effects is already in the range of 4 ppm.

iii. "insufficient histopathology and necropsy". Although these may be considered deficiencies in terms of current standards, TB-1 does not consider the lack of more detailed histopathology and necropsies in this study to be serious enough to warrant repetition of the study. This is particularly true in this study because a NOEL for systemic toxicity has already been established

in other rat studies at 4 ppm (which is considerably below 25 ppm, the lowest dose tested in this study) and systemic effects noted in this study, if any, would not affect the assignment of the NOEL of 4 ppm for systemic toxicity.

iv. "unknown clinical observation intervals". The "unknown clinical observation intervals" is considered a deficiency in the reporting of the study but not serious enough to warrant the repetition of the study.

v. "too few males". If the males in the groups dosed with 50 and 100 ppm were to be combined there would be a single group of 20 male rats dosed with at least 50 ppm of lindane. Such a combining of groups provides a way to justify that a sufficiently large group of males was tested at and above a certain dose level. TB-I does not consider the low number of males (10) in each group to be of sufficient concern to warrant a repetition of the study.

IN CONCLUSION, TB-I does not concur with the CDFA that a multi-generation reproduction study is a DATA GAP for lindane.

[Note: Mr. Roger Gardner, TB-I, who reviewed the reproductive and teratology studies for the Registration Standard for Lindane kindly assisted in the preparation of the above comments.]

2. Mutagenicity: Chromosome mutation (aberration).

First of two studies listed by CDFA

[Study identification: In Vivo Sister Chromatid Exchange Assay in CF-1 Mouse Bone Marrow Cells with Lindane (oral application). Research & Consulting Company 6/20/84.]

A. The following are the comments made by CDFA:

"Lindane (99.8%) tested at 0, 2, 10 and 50 mg/kg in male and 0, 1.6, 8 and 40 mg/kg in female in oleum arachidis by single oral gavage (10 ml/kg) in Charles River CF-1 mice; doses were 1/75, 1/15, and 1/3 of the LD_{50}; 5 mice/sex/group; BUdR tablets implanted 2 hours after dosing; mice sacrificed 24 hours after dosing; 30 metaphases/animal scored; the high dose male SCE frequency was statistically higher than control frequency while the high dose female SCE frequency was statistically lower than control frequency; the averaged frequency was not different from the controls; NO ADVERSE EFFECTS; UNACCEPTABLE-dose levels not high enough. Originally considered acceptable-Gee 9/25/85; second review considered unacceptable-Gee & Choy 12/10/86; review of C.I.E.L. rebuttal of 9/21/88, still unacceptable-Davis & Gee 9/16/88."

B. TB-1 response.

TB-I has already indicated that this study has deficiencies. For example, the one liner for this study indicates:

"Negative in CF-1 males and females for sister chromatid exchanges at single oral dose up to one third of reported LD_{50}. However, insufficient dosage and sampling sizes employed; no clinical or cytotoxicity at any dose".

The CDFA refers to a letter dated 7/1//85 from the EPA product Manager which indicates that the CORE Grade for this study has been changed to "Acceptable". Dr. Irving Mauer, TB-1, who is responsible for reviewing the mutagenicity and genotoxicity data base for lindane is unaware of this letter and maintains that the above study is Unacceptable because of the deficiencies listed in the one liner.

Second of two studies listed by CDFA.

[Study identification: "In Vivo Sister Chromatid Exchange Assay in CF-1 Mouse Bone Marrow Cells with Lindane (i.p. injection)." Research and Consulting Co. (7/17/84].

A. The following are the comments made by the CDFA:

"Lindane (99.8%) tested at 0, 1.3, 6.4, and 32.1 mg/kg in oleum arachidis by a single i.p. injection (10 ml/kg) in Charles River CF-1 mice; doses were 1/75, 1/15 and 1/3 of the LD_{50}; 5 mice/sex/dose group; BUdR tablets implanted 2 hours after dosing; mice sacrificed 24 hours after dosing; 30 metaphases/animal scored; increases in SCE frequency in female mice at 32.1 mg/kg (1/3 of LD_{50}); POSSIBLE ADVERSE EFFECT; UNACCEPTABLE pending historical control data. Originally considered acceptable-Gee 9/25/85; second review considered unacceptable-Gee & Choy 12/10/86; review of C.I.E.L. rebuttal of 12/21/87, still unacceptable-Davis & Gee 9/16/88."

B. TB-1 response:

According to Dr. Irving Mauer this study was conducted and reported in a manner that is consistent with the current OECD/EPA Guidelines.

IN CONCLUSION. TB-I does not concur with the CDFA that a Data Gap exists for a chromosome mutation (aberration) study. The second of the two studies listed above fulfills the Data Gap.

3. Mutagenicity: DNA Damage.

The following is a general comment made by the CDFA:

"The three submissions in this category are journal articles which are considered supplemental because they were not intended to be guideline studies. Two are unscheduled DNA synthesis assays in which lindane was tested in a battery of pesticides. The third article demonstrates covalent binding of lindane to DNA. Although the authors argue that this binding cannot explain the oncogenicity of lindane, it does pose a possible source of mutation, which is important in its own right."

No other specific adverse comments were made by the CDFA on any of the three studies (journal articles). The comments on each article are listed below:

Study identification: "Chemically-induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison with Bacterial Mutagenicity Using 218 Compounds" by Probst, McMahon, Hill, Thompson, Epp and Neal, Lilly Research Laboratories, Indianapolis (Environmental Mutagenesis 3:11-32, 1981).

Comments made by CDFA:

"NO ADVERSE EFFECT-Lindane was found negative in both an unscheduled DNA synthesis assay in primary rat hepatocyte cultures and a modified Ames assay. The unscheduled DNA synthesis assay was done with 100 nmoles of lindane per ml. SUPPLEMENTAL STUDY. Davis 9/13/88."

Study identification: "The Relevance of Covalent Binding to Mouse Liver DNA to the Carcinogenic Action of Hexachlorocyclohexane isomers". Institute of Toxicology, ETH and University of Zurich, 1983.

Comments made be CDFA:

"NMRI, CF1, and B6C3F1 male mice were dosed with alpha, beta, delta, or gamma (lindane) hexachlorocyclohexane by oral gavage. Covalent binding to liver DNA was found at equivalent levels for all isomers except beta which had little binding. Gamma isomer binding was equivalent in all three mouse strains. DNA synthesis was also stimulated. The authors conclude that the known liver oncogenicity of hexachlorocyclohexane isomers, including lindane, is best explained by a nongenetic mechanism. POSSIBLE ADVERSE EFFECT-Covalent binding of lindane to DNA. SUPPLEMENTAL STUDY. Davis 9/13/88."

Study identification: "Pesticide induced DNA Damage and Its

Repair in Cultured Human Cells" by Ahmed, Hart, & Lewis, Ohio State University, (Mutation Research 42:161-174, 1977)."

Comments made by CDFA:

"Lindane was one of 13 pesticides tested for unscheduled DNA synthesis (autoradiography) in SV-40 transformed human fibroblasts (cell line VA-4) +/- S9. NO ADVERSE EFFECT-Lindane was negative with 8 hours at 1 and 1000 uM. SUPPLEMENTAL STUDY. Davis 9/13/88."

TB-1 CONCLUSION.

Health Effects Division policy considers that mutagenicity studies that are published in the literature as journal articles may be used to satisfy the testing requirement for mutagenicity and/or genotoxicity at the discretion of the reviewers and in concurrence with the Branch mutagenicity/genotoxicity specialists. Since no specific or detailed adverse comments were made by CDFA regarding these studies and no indications that lindane caused direct DNA damage were raised in the unscheduled DNA synthesis studies, TB-1 has accepted these studies and does not consider that there is a Data Gap for a DNA damage study.

The observation that lindane binds to DNA is an interesting observation but does not in itself have regulatory significance.

[Note: Dr. Irving Mauer, TB-1, who was responsible for assessing the mutagenicity data base for the Toxicology Branch Chapter of the Registration Standard for Lindane has approved of the above comments.]