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

004524

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 5 1985

MEMORANDUM

SUBJECT: Lindane - [A] Appraisal of CIEL Response, January 16, 1985, in re: CIEL Mutagenicity Tests. [B] Review of mutagenicity assay (i.p. in vivo SCE) in response to PD-4. Acc. #254504. Reg. #359-686, 359-687, 2117-3, 45983-1. Caswell 527

FROM: Irving Mauer, Ph.D., Geneticist
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TO: George T. LaRocca, PM 15
Registration Division (TS-767 C)

THRU: Jane E. Harris, Ph.D., Head
Section VI, Toxicology Branch/HED (TS-769C)

[A] By letter of January 16, 1985 with four enclosures (Exhibits 1 through 4) from McKenna, Conner and Cuneo, the Centre International d'Etudes du Lindane (CIEL) has responded to EPA's review and evaluation of two mutagenicity assays sponsored by CIEL, which were judged UNACCEPTABLE, viz:

1. "Mammalian Cell (V79) Mutagenicity Test on Lindane," (Report [illegible]), performed by [illegible], Ph.D., Professor of Toxicology, Director, Institute of Toxicology, University of Mainz, [illegible] Strasse 67, [illegible] Mainz (reported as NEGATIVE).

2. "In vivo Sister Chromatid Exchange Assay in CF1-Mouse Bone Marrow Cells with Lindane," performed by Research and Consulting Company AG (Report #RCC-025765), 4452 Itingen, Schweiz (reported as NEGATIVE).

EPA judged the first assay (in vitro gene mutation in V79 cells) inadequate due to the following exceptions to agreed upon protocols of June 6, 1983.

(i) Hepatic microsomes should have been prepared from male CF-1 mice, not the reported CD-1's, since the increased tumor frequencies reported in the Ivorge & Walker study (1973) occurred in the former;

(ii) If the first assay is negative, a repeat experiment is required.

As explained by Professor F. Czech (November 8, 1984, transmitted by McKenna et al. as Exhibit 4), the appropriate metabolic activation system (from CF-1 mice) was indeed used, and the "CD-1" was a typing error in the report submitted (the invoice for the animals clearly indicates receipt of CF-1's). Additionally, EPA accepts the repeats of the activated assays as consistent with the agreed Study Plan. Therefore, with resolution of these objections, this study is ACCEPTABLE.

The second study (in vivo sister chromatid exchange assay) has also been upgraded to ACCEPTABLE because the deficiencies initially cited, namely: (1) "Insufficient dosage was administered, as indicated by the lack of animal and/or target tissue toxicity, i.e., no evidence was presented that the test compound was absorbed in effective concentrations to affect bone marrow cells"; (2) "Thirty cells per animal is an insufficient number of cells analyzed"; (3) "A minimum of 7 animals per dose, and analysis of 100 cells per animal, are recommended by the OECD guidelines cited by the author (OECD Short-Term Toxicology Group, Draft No. 14, U.S. proposal October, 1982)" are resolved by consideration of the January 16, 1985, CIIT rebuttals, as well as previously agreed-upon protocols.

A point-by-point appraisal of this rebuttal follows:

Registrant Rebuttal: "The Agency's first concern with the findings of this study is that it was not performed in 'accordance with OECD guidelines previously cited by the author...' With the exception of four EPA suggestions, each of which was considered by the laboratory in preparing the final protocols and which do not undermine the Agency's present criticism, the protocols for the SCE assay were approved by EPA in the Agency's letter of August 15, 1983. (Exhibit).) The protocols approved by EPA in 1983 reference Draft No. 14 of the OECD short-term toxicology protocols (1983). Since then, however, OECD has issued revised in vivo SCE guidelines. (Exhibit 2.) CIIT's SCE assay meets or exceeds all of the requirements of the revised and potentially-effective OECD SCE guidelines."

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EPA Response: The Agency accepts this rebuttal, in noting the following: (i) "Exhibit 1" refers to a cover letter sent by FMC George Lanocca, with an attachment indicating a number of apparent deficiencies of the then proposed protocol. (ii) Further, in a letter sent to EPA (dated August 16, 1984), CIEL was cited as having submitted two SCE studies (one oral, the second i.p.), dated June 20, 1984, and July 17, 1984, both conducted at BOC. Toxicology Branch, however, received only the oral mouse study (BOC Project 025705, dated June 20, 1984, EPA Acc. #254504), and that was the study subject of the attached review and evaluation."

Registrant: "The Agency's second criticism of the lindane SCE assay is that an insufficient dosage of the test article was administered to the CF-1 mice. Page 8 of the EPA-approved protocol, however, stated the dosages that would be administered to the test animals, including a high dose that was 1/3 of the acute LD_{50}. (Exhibits 2, 3). As Dr. M. Herbst of Research and Consulting notes in his response, 'the high dose lie[s] within the acute range (including death and target cell toxicity. (Exhibit 3.)"

EPA Response: The Agency accepts this rebuttal.

[R] Tox. Branch has recently received the second sister-chromatid exchange assay (intraperitoneal injection) mentioned in the CIEL letter of August 16, 1984 (BOC Project 025714, dated July 17, 1984), and the review of this study is attached to this memo. This study is judged ACCEPTABLE.

[illegible], however, [illegible] of this memo, and slipsheeted into review for the i.p. SCE study.

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TOXICOLOGY BRANCH: DATA REVIEW

<u>CHEMICAL:</u> Lindane

Caswell: 527
EPA Chem: #009001

<u>STUDY TYPE:</u> Mutagenicity: Sister-chromatid exchange in mice (*in vivo* SCE).

<u>CITATION:</u> In vivo Sister Chromatid Exchange Assay in CFl-Mouse Bone Marrow Cells with LINDANE (Interperitoneal Injection).

<u>ACCESSION NO./MRID NO.:</u> 24504/NA

<u>SPONSOR/TESTING LAB:</u> Centre Internationale d'Etudes du Lindane (C.I.E.L.), Bruxelles/
Research & Consulting Company AG (RCC).
4452 Itingen/Schweiz

<u>STUDY NO./DATE:</u> RCC Project 025716/July 17, 1984

<u>TEST MATERIAL:</u> Lindane (technical), batch/lot no. 83/33, 99.8 percent ai, a white powder suspended in arachis oil for ip administration.

<u>PROCEDURES:</u> A copy of the procedures, performed according to referenced standards, is attached to this review. Briefly, the test article (in arachis oil) was administered ip to groups of six male and six female CFl mice (10 ml/kg bw oil) at single dose levels of, respectively, 0 (vehicle control), 1.1, 5.6 and 22.4 mg/kg, respectively 1/75th, 1/15th and 1/3rd the LD50, 2 hr after implantation of 50 mg tablets of bromodeoxyuridine (BrdU). Cyclophosphamide (CP, 10 mg/kg) served as the positive control.

<u>RESULTS:</u> No toxicity was reported in any treated group. Sampling of bone marrow cells from 5 animals per group (30/animal) 24 hr following single doses of test compound revealed slight but statistically significant increases over all controls in SCE frequency in all female test groups (but not in males) without a dose-related trend (1.29, 1.82, 2.12 per cell, compared to 1.56 SCE/cell for controls). When the results from males and females were pooled (no calculations were provided however) only the value of high-dose treatment remained significantly different from controls. The positive control (treated with CP) responded appropriately, with

clearly significant increases in SCE (5x oil controls). The authors also determined that the female control group value (1.56 ± 0.089 SCE/cell) was significantly lower than the male group (1.86 ± 0.107).

CONCLUSIONS:

The authors concluded that "...under the experimental conditions reported during this in vivo chromatid exchange assay with LINDANE, no chromosome damage was observed in the bone marrow cells of the animals treated at 1.3, 6.4 and ≥12.1 mg/kg body weight. Thus, no potential chromosome mutagenic activity was evident in this in vivo test...."

TR EVALUATION:

This study is ACCEPTABLE.

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Lindane toxicology review

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