



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

004524

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JA 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. #155-686, 359-687, 2117-3, 40081-1. Canwell 527

FROM: Irving Miler, Ph.D., Geneticist
Section VI, Toxicology Branch/NEB (TS-7693)

TO: George I. LaRocca, PM 15
Registration Division (TS-7670)

THRU: Jane E. Koppa, Ph.D., NEB
Section VI, Toxicology Branch/NEB (TS-7693)

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

1. "Mammalian Cell (V79) Mutagenicity Test on Lindane," (Report #R-54-VI-21), performed by Peter Cuneo, Ph.D., Professor of Toxicology, Director, Institute of Toxicology, University of Mainz, where published release 67, M-6502 Mainz (reported as NEGATIVE).
2. "In vivo sister chromatid exchange assay in C3H mouse bone marrow cells with lindane," performed by Research and Consulting Company AG (Report #RCC-025765), 4032 Itingen, Schweiz (reported as NEGATIVE).

EPA judged the first assay (in vitro gene mutation in v79 cells) inadequate due to the following exceptions to official protocols of June 6, 1983:

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- (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 George & Walker study (1973) occurred in the former;
- (ii) If the first assay is negative, a repeat experiment is required.

As explained by Professor F. Oesch (November 2, 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) "Inefficient 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 burning (OECD short-term Toxicology Group, Draft No. 14, U.S. proposed October, 1980)" are resolved by consideration of the January 14, 1985, CRLA remittals, as well as previously agreed-upon protocols.

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

Registration Remittal: "The Agency's first concern with the in vivo SCE assay study is that it was not performed in accordance with OECD guidelines previously cited by the Agency..." With the exception of four EPA suggestions, each of which was considered by the laboratory in preparing the final protocols and which do not concern the Agency's present criticism, the protocols for the SCE assay were approved by EPA in the Agency's letter of August 18, 1983. (Exhibit 1.) The protocols approved by EPA in 1983 referenced Draft No. 14 of the OECD short-term toxicology protocols (1980). Since then, however, OECD has issued revised in vivo SCE Guidelines. (Exhibit 2.) CRLA's SCE assay meets or exceeds all of the requirements of the revised and currently-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 PM George Iannone, 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 SOE studies (one oral, the second i.p.), dated June 20, 1984, and July 17, 1984, both conducted at ROC. Toxicology Branch, however, received only the oral mouse study (ROC Project 025705, dated June 20, 1984, EPA Acc. #251504), and that was the study subject of the attached review and evaluation.*

Registrant: "The Agency's second criticism of the lindane SOE assay is that an insufficient dosage of the test article was administered to the CF-1 mice. Page 2 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 LD₅₀. (Exhibits 2, 3). As Dr. M. Warner of Research and Consulting notes in his response, 'the high dose [is] within the acute range (including death and target cell toxicity. (Exhibit 3.)'"

EPA Response: The Agency accepts this rebuttal.

(ii) Tox. Branch has recently received the second intraperitoneal exchange assay (intraperitoneal injection) mentioned in the CIEL letter of August 14, 1984 (ROC Project 025716, dated July 17, 1984), and the review of this study is attached to this memo. This study is judged ACCEPTEABLE.

Note, however, III of this memo, and attached data review for the i.p. SOE study:

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TOXICOLOGY BRANCH: DATA REVIEW

CHEMICAL: Lindane

Caswell: 527
EPA Chem: #109001

STUDY TYPE: Mutagenicity: Sister-chromatid exchange in mice
(in vivo SCE).

CITATION: In vivo Sister Chromatid Exchange Assay in C3H-
Mouse Bone Marrow Cells with LINDAKE (Intraperitoneal
Injection).

ACCESSION NO./ABID NO.: 24504/LA

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

STUDY NO./DATE: RCC Project 025714/July 17, 1984

TEST MATERIAL: Lindane (technical), batch lot no. 21/11,
99.8 percent ai, a white powder suspended in arachis oil for
ip administration.

PROCEDURES: 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 C3H mice (10-12 wk old) at
single dose levels of, respectively, 0 (vehicle control),
1.1, 4.4 and 22.1 mg/kg, respectively 1/75th, 1/15th and 1/10th
the LD50. A 100% effect implication is 50 mg/kg tablets of
Dieldrin, 100% (RCC). Cytogenetic analysis (CF, 10 mg/kg)
acted as the positive control.

RESULTS: No toxicity was reported in any treated group.
Sampling of bone marrow cells from 5 animals per group (20/
animal) at the following single doses of test compound revealed
slight but statistically significant increases over all
controls in the frequency in all female test groups (but not
in males) without a dose-related trend (1.29, 1.82, 2.12
and cell, compared to 1.56 for all four controls). When the
results from males and females were pooled (no calculations
were provided however) only the value of highest treatment
remained significantly different from controls. The positive
control (treated with CF) responded appropriately, with

clearly significant increases in SCE (9x 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 ± 0.207).

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 312.1 mg/kg body weight. Thus, no potential chromosome mutagenic activity was evident in this in vivo test...."

TE EVALUATION:

This study is ACCEPTABLE.

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Lindane toxicology review

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