CASWELL FILE

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 359-686 - Lindane: Review of protocols for 90 day dermal toxicity studies in rats and rabbits and 14 week aerosol inhalation study in mice.

TOX CHEM No. 527
TOX Project No. 1737
Record No. 173085

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Background

The Centre International d'Etudes du Lindane (CIEL) through their attorneys the McKenna, Conner and Cuneo Co. have submitted protocols for certain studies required in the DATA-CALL-IN Notice issued January 23, 1986. These protocols were reviewed by Toxicolgy Branch (TB) and the comments below are being provided.

Toxicology Branch Comments

CONCLUSION. TB has made many comments and suggestions related to the study protocols. Thus, the protocols should be rewritten and resubmitted for review by TB.

OTHER COMMENT. In the accompanying letter, reference was made that a rat inhalation study has already been submitted. The registrants should be advised that TB has not concurred with the conclusions of the study regarding assignment of the NOEL and LEL

as presented. Additional information related to the potential effects of lindane on the bone marrow noted in this study have been requested (refer to J.D. Doherty memo dated April 25, 1986).

The identity of the protocols are listed as follows:
List of protocols presented:

Title	Laboratory	Number
Lindane: 90 day dermal toxicity study in the rat	Hazleton Laboratories Europe	P2979s
Lindane: 90 day dermal toxicity study in the rabbit	Hazleton Laboratories Europe	P2978s
Lindane 14-week dust aerosol inhalation study on mice	Bushy Run Research Center	86-85-80201

The following comments relate to all three studies unless otherwise indicated.

1. The kidney has been identified as a potential target organ for lindane toxicity in both rat subchronic feeding and inhalation studies. The three proposed studies must include specific tests to determine if lindane affects the functional capacity of the kidney as well as present evidence of a thorough pathological examination of this organ.

   The registrant is requested to submit their plans for determining potential effects of lindane on kidney function.

   Urinalysis should also be evaluated for all animals on the study prior to the interim, terminal and recovery sacrifices (the protocols state that for the dermal studies, urine samples will be taken only under specific circumstances).

   Extra slides (for example three slides of both the left and right kidney from each animal) of this organ should also be prepared and examined. All animals on these studies should be examined microscopically for kidney pathology.

2. The bone marrow has been implicated as a potential target organ for the toxicity of lindane based on the review of the rat 90 day inhalation toxicity study which was translated from German (refer to review by

J.D. Doherty dated April 25, 1986). Each of the three studies for which the protocols have been submitted must also include special assessments of the bone marrow content or bone marrow myelograms. The Pappenheim staining technique should be used unless justification is provided for use of some other stain.

Each study should thoroughly address the issue of potential effects of lindane on the bone marrow and for hematotoxicity. This includes bone marrow myelograms and hematology assessment for all animals at the interim, terminal and recovery sacrifice times.

3. The two dermal toxicity studies must include interim sacrifices at both 30 and 60 days. The inhalation study must include an interim sacrifice at 45 days. All three studies must include a recovery group that will be sacrificed 6 (six) weeks after the last dose application.

   Each extra group should have 10 animals per sex per group.

   The provision for inclusion of extra animals for a 6 week recovery period was included in the DATA-CALL-IN notice sent to registrants of lindane on January 23, 1986. The requirement for the inclusion of the interim sacrifice groups was made after review of the 90 day inhalation study in attempts to determine the time of onset for the effects of lindane on the kidney and bone marrow. Determining the time of onset is important in utilizing the study data for determination of the Margin of Safety for the various uses of lindane.

4. All three studies must include analysis of the blood for lindane content. The laboratory should consider available pharmacokinetic data with lindane and try to analyse the blood when the lindane concentration is expected to be optimal. Assessments for blood content of lindane should be made at each of the interim sacrifices and at termination.

5. For the dermal toxicity studies, the vehicle to be used with the test material was not identified. This information should be provided. TB cautions the registrant that the selected vehicle (if a vehicle will be used) should be one that will not compromise the integrity of the skin such that more lindane will be absorbed. The vehicle should not also be an oil such as corn oil which will retain the lindane and result in less absorption. TB suggests that if possible the lindane powder might best be applied directly onto the skin and kept in place with a system of bandages. If this system is used the bandaging system must use a fine gauze such that the powdered lindane cannot easily become enmeshed in the gauze and not be available for absorption.

6. All three studies must adhere to the recommendations in the EPA Guidelines as of October 24, 1984 (49 FR 42856). Exceptions to these recommendations must be justified.

7. For each study the test material must be clearly identified as being 99.6% gamma isomer and as being a technical lindane product. The analytical concentration of lindane in the dosage form must be determined and its stability in the formulation monitored. If lindane powder is used for the dermal toxicity studies, its concentration must be presented together with storage and stability conditions.

Comments on the individual studies.

A. The rat dermal toxicity study.

1. The Wistar rat strain which previously showed kidney and bone marrow toxicity in both dietary and inhalation studies should be used.

2. Extra groups (10 rats/sex/group) for each interim sacrifice and for the six week recovery phase must be included.

3. Clinical pathology should be performed on the rats sacrified at each of the interim, terminal and recovery sacrifices of each study.

   i. Hematology should also include hematocrit.

   ii. Bone marrow myelograms should be made for each sacrifice time for all animals on the study.

   iii. The hematology section (in either the results or the discussion of the study report) should include a discussion that shows justification that the blood and bone marrow were thoroughly assessed to investigate possible effects of lindane as indicated by the 90 day inhalation study which showed effects of lindane on bone marrow myelograms.

4. Urinalysis must be included and special plans devised to assess kidney function must be presented. Urinalysis and kidney function assessment must be made just prior to sacrifice for the interim, terminal and recovery groups.

5. The organs to be weighted should also include at least the spleen, thymus and ovaries. All of the organs as recommended by the EPA Guidelines should be included.

6. Histopathology for the low and mid dose animals should include the following list of organs:

lungs
liver
kidneys
sternum (with myelogram of bone marrow)
femur " " " " "
testis
ovaries
spleen
thymus

(and any organ or tissue which might show a secondary effect resulting from either kidney effects or hematotoxicity).

B. The rabbit dermal toxicity study.

This protocol is similar to and is from the same laboratory as the above rat study protocol. Thus, all of the above comments (except for the selection of the strain of rat) made for the rat study apply. The test animal, New Zealand White rabbit, is considered appropriate.

C. Mouse inhalation study.

1. Extra groups must be included for the interim sacrifice and recovery period. The recovery period must be for six weeks. It is considered unwise by TB to have only high dose and control animals in the recovery period.

2. TB recommends that a preliminary test be run to assure that the method of generation of the atmosphere to be used will result in a respireable atmosphere of lindane. For example, the blood levels should be tested in a preliminary experiment and compared with the blood levels in the rat inhalation study. Failure to show that the lindane was absorbed into the mice may result in declaring the study a no test.

3. The method of sampling the chamber to determine the atmospheric concentration of lindane was unclear in the proposed protocol. The procedure to be used should be more specifically stated and the location of the atmosphere sampling system in relation to the breathing zones of the mice should be included.

5. The hematology and urinalysis assessments should be complete and include all animals in all groups. The registrant must devise plans for assessing kidney function.

5. All organs recommended in the EPA Guidelines should be weighted. In particular the spleen and thymus should be included.

6. The sternum and femur should be included in the list of tissues for histopathological analysis. Myelograms should be taken for these structures.

7. The organs/tissues listed under point 7 for the rat study protocol above should also be examined for the low and mid dose level groups.