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

JUL 22 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Lindane: Company meeting to discuss protocols for the rat chronic feeding/oncogenicity study.

TOX CHEM No. 527

FROM: John Doherty *John Doherty 7/16/86*
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: George LaRocca, PM 15
Registration Division (TS-767)

THRU: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769) *Budd 7/13/86*

THRU: Theodore Farber, Ph.D. *Theodore M. Farber 7/18/86*
Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Present at the meeting held at Crystal City June 26, 1986 at 10:00 AM.

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Peg McMullen and Glenn Simon, representatives for the Centre International d'Etudes du Lindane (CIEL).
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George LaRocca, PM #15
Dana Pilett, PM team #15
Ed Budd, Tox Branch
John Doherty, Tox Branch

The Product Manager is requested to forward this memo summarizing the meeting and associated intra Toxicology Branch (TB) discussions which took place following the meeting to the CIEL representatives.

The meeting was held at the request of CIEL to discuss the protocols for the rat chronic feeding/oncogenicity study. At the time of this meeting the situation with the CIEL was that the rats were already received by the testing laboratory (Hazleton Laboratories Europe Ltd.) and apparently in the acclimatization stage. The study was set to initiate feeding of the test material on approximately June 27. The CIEL had previously submitted a protocol for Toxicology Branch (TB) approval (RD due date 6/26/86). The submission also included a letter from Dr. Bernard Goldstein, a consultant, who addressed the issue of including special tests and extra animals for assessment of the hematoxicity of lindane.

At the meeting the following items were discussed:

1. Assessment of bone marrow content. J. Doherty initiated the discussion by indicating that the content of Dr. Goldstein's letter had limited usefulness because the review of the rat subchronic inhalation study (Dr. Goldstein apparently had not seen either the review or the study) indicated changes in the composition of the bone marrow. Because of the potential hematoxicity of lindane as observed in the subchronic inhalation study, TB stated that future subchronic toxicity studies should assess the bone marrow for possible changes in composition as a result of lindane toxicity.

TB advised that the Pappenheim stain should be used in staining the bone marrow smears but that if the pathologist used another stain, justification would have to be provided.

The CIEL representatives acknowledged that the bone marrow assessments would be made on all animals in the study.

2. Kidney Effects. The CIEL agreed that the kidney is a target organ for lindane toxicity and that special attention would be given to this organ in this study. J. Doherty indicated that extra slides (three per kidney) of each kidney (left and right) should be examined from each rat including those in the interim sacrifice groups.

It was also indicated by TB that special attention should be given to functional assessment of the kidney. The CIEL was requested to submit their plans for assessing the function and status of the kidneys to EPA for review. Novel approaches toward assessing kidney function in rats as proposed by the CIEL would be welcome.

After the meeting this problem of how to assess kidney function in rats was discussed with Dr. Kasza, TB pathologist, who suggested that the conventional urinalyses, if both qualitative and quantitative analyses of the urine in both the early and later stages of the study are stressed, would give data indicative of functional impairment of the kidney if present.

Note: TB recognizes that there are no generally accepted kidney function tests for use with rats and that the small volume of urine produced by this species makes conventional kidney function tests used in humans inappropriate for rats.

Other aspects of assessing the kidney effects are discussed in the following section (3) and in the AFTERWARD notes of this meeting (see below).

3. Interim Sacrifices. Further discussion concerned the interim sacrifices that would be necessary to determine if lindane causes an early effect in the bone marrow and/or kidney that is later adapted to over the course of continued feeding. Intervals suggested by TB were 45 and 90 days, 6 months and one year and at terminal sacrifice. The company objected to the 45 and 90 day sacrifices because they doubted that a toxicity response followed by an adaptation response would occur over this short time span. CIEL proposed interim sacrifices of 6 months and one year. The six month interval and not the 45 or 90 day sacrifice seemed justified according to CIEL because the subchronic inhalation study already showed the hematotoxicity and nephrotoxicity effects at this time (i.e. 90 days).

After the meeting in a discussion with Dr. Louis Kasza, TB pathologist, the issue of the appropriate interim sacrifice times was again brought up. Dr. Kasza strongly emphasized that the best way to assess for potential hematotoxicity was to include early sacrifice times. Thus, the chronic feeding study should include interim sacrifices at 45 days, 6 months and 1 year and the terminal 2 year sacrifice. In addition, the rats should be sampled for blood and smears made and evaluated at 30, 45 and 90 days, 6 months, 1 year and at terminal sacrifice.

Including the interim sacrifices at 45 days, 6 months and 1 year will require inclusion of satellite groups of at least 10 rats/ sex/group for each sacrifice time.

The interim sacrifice times of 45 days, 6 months and 1 year are also deemed to be appropriate for assessing the status of the kidney as per discussion with Dr. Kasza. At each sacrifice time, the urine should be mechanically removed from the bladder of each rat and assessed for its content both qualitatively and quantitatively.

4. Selection of the strain of rat for testing. J. Doherty advised that the strain of rat to be used for the chronic feeding study should be the same strain that was used for the 12 week subchronic feeding and subchronic inhalation studies. Dr. Simon presented a case for using the Sprague-Dawley rat (the strain already received by the testing laboratory). His reasons included that this strain was widely used for toxicity testing and much historical control data are available. It is also a strain used by some researchers for determining adverse effects on the kidney. Although TB acknowledged the remarks made by Dr. Simon, TB still thought it advisable to use the Wistar rat which is already known to be susceptible to the nephrotoxicity of lindane. If another strain were used and did not show a NOEL of less than or equal to the NOEL observed for the subchronic study, TB would have to use the NOEL for the most sensitive strain for purposes of setting an ADI. A 1000 fold safety factor might have to be used with the subchronic study and a 100 fold safety factor might be used for the chronic feeding study.

Later in the course of the meeting it was noted that a 42 day range finding study indicated that nephrotoxicity was observed in the male rats. Rats in all dosed groups were affected. The CIEL estimated the NOEL by extrapolation to be 30-50 ppm. Thus, on this basis the 2-year study would not be conducted with the most sensitive strain. This issue is further discussed under AFTERWARD below.

5. The CIEL representatives presented the results of the range finding study to Dr. Pilett for logging in and forwarding to TB.

6. Selection of dose levels. Based on the range finding study, the dose levels recommended by the CIEL toxicologists were 0, 4, 16, 80 and 400. The 400 ppm level was estimated to be the MTD.

TB will not approve of dose levels for protocols. The selection of the dose level is the responsibility of the study sponsor.

After the meeting, TB recalled, however, that the lowest proposed test dose level of 4 ppm may possibly result in kidney effects. The 12 week subchronic study had very minimal and questionable kidney effects at this level which Dr. K. Locke the original reviewer acknowledged but did not consider of sufficient magnitude to be a definite effect level. The subtle effects noted after 12 weeks of dosing at 4 ppm may be compounded by continued feeding

and the chronic feeding study may not show a NOEL. Thus, TB cautions that a dose level of lower than 4 ppm should be included in the study. The above cautionary note should be communicated to the CIEL.

7. Protocols for subchronic studies with lindane. At the meeting E. Budd also informed the CIEL representatives that the protocols for three subchronic studies previously submitted (two dermal toxicity studies, one with rats and one with rabbits, and an inhalation study with mice) would have to be revised and resubmitted by the CIEL. The original protocols had been reviewed by TB and specific suggestions for corrections would be forwarded to RD in the next few weeks.

AFTERWARD

Some of the issues raised at the meeting with the CIEL group were discussed between Dr. Theodore Farber (TB Branch Chief), Ed Budd and John Doherty at a meeting on Friday June 27, 1986 at 10 AM.

At this meeting Mr. Budd described the situation to Dr. Farber. The principle concerns which Dr. Farber had were that the most sensitive strain of rat should be used for the chronic feeding study. On the basis of available information it could not be determined that the Sprague-Dawley strain proposed for use is the most appropriate strain. Dr. Farber felt that the Wistar rat (which was used in the 12 week feeding and inhalation studies) in the absence of additional information should be used for the chronic feeding/oncogenicity study.

In the 12 week study with Wistar rats, kidney effects were observed at 20 and 100 ppm (and subtle but questionable effects were possibly seen at 4 ppm). In the range finding study with the Sprague-Dawley rats, kidney effects were observed at all dosage levels, but TB could not determine exactly what the lowest dosage level was (although it could be estimated to be 100 ppm). TB requests that RD ask the CIEL to provide the dose level that was used as the lowest test dose level for this range finding study. On this basis, the CIEL estimated that the NOEL for kidney effects in the Sprague-Dawley rat would be 30-50 ppm.

Other evidence that the Wistar rat may be a more sensitive strain is that the subchronic inhalation study with this strain had a NOEL of 0.01 mg/kg/day, or 30 fold lower than that observed in the 12 week feeding study.

Dr. Farber also suggested that a specific "water loading" kidney function test be considered for inclusion in the chronic feeding study. This test is a renal concentrating test and the following reference provides some of the details of an adaptation of this test and presumably other references for the test in adult rats: Kavlock and Gray, Biology of the Neonate 41:279-288 (1982). TB will provide additional information on this test in its response to the rat chronic feeding/oncogenicity study protocol.

Dr. Farber further suggested that TB provide the CIEL with the latest position paper on the Maximum Tolerated Dose MTD. He also indicated that the MTD might best be estimated from the 90 day subchronic study with the Wistar rat. End points that should be considered in the MTD level should be a 10% decrease in body weight and/or some other life threatening histopathological effects. A copy of the most current MTD position paper is attached.

Dr. Farber also suggested the possibility of including in the study extra groups of rats for each dose group to be treated with lindane for 18 months and then left untreated for the final six months. For example these rats would be recovery groups.

As a follow up to the meeting with Dr. Farber, Ed Budd notified the Product Manager (Mr. LaRocca) with a request to contact the CIEL to advise them of Dr. Farber's concerns regarding the selection of the strain to be used for this study.

In conclusion, TB does not recommend that the CIEL initiate the study at this time using the Sprague-Dawley rat.

The CIEL should reassess the selection of the strain, ^{and} present their study protocol which should include their approaches to assessment of kidney function and the times of interim sacrifices. All other aspects of the study protocol should also be submitted.