CASWELL FILE

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

APR 21 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. NO. 359-686. Lindane: Review of registrants comments on the protocols for the 5 day pilot inhalation study and 14 week inhalation study with lindane.

TOX. CHEM. No.: 527
TOX. PROJECT No.: 7-0590
Record No.: 193465

FROM: John Doherty 4/20/87
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: George LaRocca
Product Manager #15
Registration Division (TS-767)

THRU: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769) 4/20/87 4/21/87

Toxicology Branch (TB) previously reviewed protocols for a 5 day pilot inhalation study (J. Doherty review dated Jan. 16, 1987) and for a 14 week inhalation study (J. Doherty review dated Jan. 27, 1987). In these protocol reviews several procedural questions were raised by the TB reviewer to which the registrants (Centre International d'Etudes du Lindane, CIEL) representatives (Dr. A. M. Blacker and Dr. G.S. Simon) are now responding. The following is an item by item discussion of the issues raised.

A. Comments on the 5-day pilot study.

1. Issue: Submission of the results of the pilot study regarding blood sampling, urinalysis and bone marrow smears to EPA for evaluation.

   TB was trying to be helpful to both the CIEL and the testing laboratory by suggesting that these procedures when worked out in the pilot study be submitted to the Agency for review. If, however, the CIEL and the testing laboratory are confident that the procedures resulting from information gained in the pilot study will be fully satisfactory for use in providing the Agency with an acceptable 14 week inhalation study, they are welcome to proceed with the longer study at their discretion without additional delay.

2. Issue: Method of generation of the aerosol containing lindane and other items regarding exposure.

   TB agrees that there is little information on the particle size, impaction and distribution available regarding the mouse but there is some data available. For example, Schlesinger (J. Tox. Env. Health 15:197, 1985) reports that particles in the range of 1-5 um are deposited with larger sizes in this range being more poorly deposited than the smaller sizes. Thus, the target range for the particles generated for the mouse study should include that most of the particles are < 5 um in diameter.

   TB's original comment that "exposure levels based on the amount of lindane in the blood should be in the same range as in the 1983 rat inhalation study" requires additional explanation on the part of TB. The registrants have made a good point in their comments with regards to possible species differences in both pharmacokinetics and susceptibility. TB's original intention for this requirement was that the rat blood levels be used as a simple guide or index. If the mouse is indeed much more susceptible, TB would readily accept the study being conducted at lower test levels and blood levels of lindane. The registrant would then have the responsibility of determining the relevant NOELs for this species and this species may then be used in determining the Margin of Safety. In the case where no toxicity is seen in the mouse study and the atmospheric and blood levels were the same or higher than in the rat, than the rat would be used in determining the Margin of Safety.

   Overall, the study should be conducted with toxicity responses as the more meaningful endpoint. The blood levels of lindane must still be determined to assure that the mice absorb the material.

3. Issue: Urinalysis.

TB strongly recommends that attempts to do a "water loading" procedure prior to urinalysis be included in the mouse pilot study to determine if this type of test can be conducted in this species.

4. Issue: Lindane metabolites in urine.

The urine does not have to be assessed for lindane metabolites provided that the blood is assessed for lindane.

B. 14 Week Inhalation Study.

1. Issue: Submission of the results of the 5-day pilot study.

Refer to point 1 under part A preceding.

2. Issue: Aerosol generation method, blood levels of lindane and validity of the study.

The method for generation of the aerosol is at the discretion of the study sponsor and testing laboratory.

It is very desireable to have a dose related increase in blood levels of lindane corresponding with the atmospheric levels of lindane for this study. Should the results of the blood analysis for lindane fail to show a dose related increase with increases in atmospheric concentrations of lindane then the study can still be valid if the registrants provide a satisfactory explanation based on pharmacokinetics and other relevant data. Such an explanation must be accompanied by a detailed account of the known pharmacokinetics of lindane in the mouse and compared to the rat which does show a dose related increase in blood levels of lindane. Appropriate references and numerical data should be provided.

3. Dose levels. Toxicity vs. Blood Levels.

Toxicity responses should be the more critical criterion in selecting exposure concentrations. In the event that no toxic responses are evident at the highest possible atmospheric level of lindane (which can produce respireable particles), the blood levels of lindane will play an important role in determining the validity of the study.

[Note: Revised protocols were not included in the submission package.]

TB has the following comments regarding the 14-week inhalation study.

Six kidney slides from each mouse should be prepared, three from each kidney. All of the mice on the study should have their kidneys examined. The pathologist should be familiar with the types of pathology noted in the rat subchronic inhalation and feeding studies and be prepared to use alternate stains if necesary to assure the Agency that the 14 week inhalation study demonstrates a NOEL for any kidney or other organ/tissue lesion or possible lindane effect.

RESPONSE TO EPA'S COMMENTS ON LINDANE INHALATION TOXICITY STUDY IN MICE

5-Day Pilot Inhalation Study

1. EPA's Comment: "Since this protocol is exploratory, the procedures to be used for sampling blood for lindane determination, analyses of the urine, and the procedure for preparing bone marrow smears for myelograms, as well as the results of all these evaluations, should be included in the final report on the pilot study, which should be sent to EPA for review prior to initiation of the 14-week study."

Response: EPA does not state their reasons for the above recommendation. The pilot study is not intended to fulfill any testing requirement for lindane. Its purpose is to validate nonroutine procedures to be employed in the 14-week study; i.e. quantitation of lindane in serum, clinical chemistry measurements in urine, myelograms. Since the mouse is the test species, the laboratory must also determine if sufficient sample volume for measurements in blood and urine will be obtained from individual animals. With the exception of blood samples collected from lindane-exposed mice for quantitation of serum lindane concentration, all procedures will be conducted on mice not exposed to lindane. Thus, no treatment-related effects will be observed in the pilot study.

Almost one year has already been spent in reviewing protocols for the inhalation work. An Agency review of the pilot study will further delay initiation of the definitive study. The overall design of the 14-week study will not be affected by the pilot work. Bushy Run Research Center is an experienced and qualified laboratory whose purpose in the pilot study is to assess the validity and feasibility of the nonroutine procedures proscribed for the 90-day study. EPA's review of the pilot work is not justifiable. The pilot study does not fulfill any EPA requirement and is being conducted to familiarize laboratory personnel with nonroutine procedures. Thus, this work does not need to be submitted to the Agency for their review.

2. EPA's Comment: "The method for generation of the aerosol must result in particles that are of a respirable size and are absorbed by the mice. Assessment of the blood levels of lindane will assure that lindane was actually absorbed. Note that exposure levels based on the amount of lindane in the blood should be in the same range as in the 1983 rat inhalation study, especially since the special type of generator that was used in that study will not be used in this proposed study."

...continued...

Response: We agree the particles should be of a respirable size. However, the EPA has not indicated the particle size they consider to be respirable for mice. Unlike the rat, the mouse is not routinely utilized for inhalation toxicity studies. To our knowledge, extensive studies of its respiratory tract with respect to particle size impaction and distribution do not exist, as they do for rats. EPA Pesticide Assessment Guidelines state, "For man, the inhalable diameter is considered here as 15 micrometers or less." Generally, in rat inhalation studies, the acceptable inhalable diameter is considered to be 10 microns or less with 80% of the particles being of this diameter. Are these criteria acceptable for a mouse study?

We also agree that proof of absorption is necessary. However, the approach that exposure concentrations should produce blood levels in the same range for the mouse as in the rat inhalation study is inappropriate for several reasons:

A species difference in sensitivity to lindane-induced toxicity might exist, and thus, obtaining similar blood levels might not be practical or even possible. Extreme examples of this difference would be either no toxicity or lethality could occur in the mouse at blood levels similar to those recorded as being slightly toxic to the rat. Another more probable situation is that lindane pharmacokinetics could differ significantly between the mouse and rat. If mouse blood becomes saturated at a much lower lindane concentration than the rat, similar blood levels cannot be achieved in both species. EPA's recommendation assumes that systemic toxicity is a direct and sole function of blood levels, and that in any species, a given blood level will produce identical effects. Clearly this is not so. If it were, extrapolations from animals to humans would be far easier.

Further, EPA's rationale that exposure concentrations should be based upon blood levels does not seem to be entirely consistent with their Pesticide Assessment Guidelines (Subdivision F) which states, "The highest concentration should result in toxic effects..." In our opinion, the more logical approach is to comply with the EPA Guidelines by demonstrating toxicity and seeing at what blood concentration the toxicity occurs. Lindane would be quantitated in the serum to prove absorption and to allow for comparisons across several concentrations with the previously conducted inhalation study in rats.

3. EPA's Comment: "The urinalyses aspect of the pilot study does not include any special procedures such as food and water deprivation prior to testing, administration of water by gavage prior to collecting the urine, or the use of individual animal metabolism cages for collecting urine. Will any such procedures be attempted in this pilot study?"

...continued...

Response: Water loading and deprivation have not been specifically recommended or suggested by the EPA for the definitive inhalation study. These procedures are difficult to conduct in the mouse due to its small size and will not be included in either the 5-day or 14-week studies. The suitability of using individual metabolism cages for urine collection will be evaluated in the pilot study.

4. EPA's Comment: "There appears to be no provision in the protocol for assessing the various excretory products in the urine. Is urinalysis limited to volume, sodium, potassium, chloride, creatinine, and quantitative protein determinations, or will metabolites of lindane also be assessed?"

Response: As stated in the protocol, one of the purposes of the pilot study is to evaluate "appropriate procedures for analyzing various excretory products in the urine." In this context, the "excretory products" to be measured are sodium, potassium, chloride, creatinine, and protein concentrations. These measurements are not routinely conducted on mouse urine and thus, procedures must be validated prior to initiation of the definitive study.

Quantitation of lindane metabolites in the urine will not be conducted and is not feasible due to the small amount of urine produced by a mouse. The urinary parameters already proposed to be measured will require most of the urine sample. In addition, the inhalation study is being conducted to assess lindane toxicity and not lindane metabolism.

5. EPA's Comment: "Please justify the use of Prussian Blue stain for the bone marrow rather than the more widely used Wright's stain. Also, why are bone marrow samples being evaluated for Papenheimers bodies? The bone marrow should be assessed for other changes as noted in the 1983 rat inhalation study."

Response: In the first review of the 14-week inhalation study protocol, EPA indicated that the Pappenheim staining techniques should be used for bone marrow myelograms. (This stain was employed in the rat inhalation study). Apparently, we misunderstood EPA's recommendation and proposed to stain for Pappenheimer bodies. This misconception has now been resolved, and, if acceptable to the EPA, Pappenheim stain will be used for the bone marrow myelograms. A complete evaluation of the myelograms will be conducted. Bone marrow will not be evaluated for Pappenheimer bodies using Prussian Blue stain.

...continued...

14-Week Inhalation Study

1. EPA's Comment: "The results of the 5-day pilot study, together with plans for using the information (including procedures) generated from this study should be submitted for Agency review prior to initiation of the 14-week study. The Agency reserves its comments on the special aspects of this study to assess for kidney and bone marrow effects pending review of the results of the 5-day study."

Response: Please refer to our previous comments on the Agency's review of the pilot study. To reiterate, in the pilot study, most of the procedures will be conducted on mice not exposed to lindane. Thus, no range-finding or preliminary toxicity data will be available for review. The pilot study is intended to validate nonroutine laboratory procedures to be used in the definitive study and does not fulfill any testing requirement for lindane. Thus, we feel an Agency review of the pilot study is neither justified or necessary.

2. EPA's Comment: "No rationale for the selection of the method of generation of the aerosol was included in the protocols for the 5-day or 14-week inhalation studies. Since the method is not the same as that used for the 1983 90-day rat inhalation study, it is essential that blood level of lindane be determined. The study will not be considered a valid test if no lindane is detected in the blood or if there is no dose-related increase in lindane in the blood. Uniformity of atmospheric generation and particle size should be assured by frequent monitoring."

Response: The method selected for aerosol generation is widely used and accepted by experts in inhalation toxicology. As stated in the protocol, atmospheric concentrations will be measured four times per day. Particle size analysis will be conducted once per day from each chamber.

We agree that lindane should be quantitated in the serum to demonstrate proof of absorption. However, to state that the validity of the study is dependent upon evidence of a dose-related increase of lindane in the blood is not reasonable. Lindane pharmacokinetics might differ significantly between the mouse and rat. Mouse blood could become saturated at exposure concentrations that do not produce toxicity. A dose-related increase of lindane in the blood would then require lower exposure concentrations. Does the EPA prefer to see a dose-related increase in blood levels or a dose-related increase of toxicity? In our opinion, the more prudent approach is to base exposure concentrations on toxicity and not blood levels.

...continued...

3. EPA's Comment: "The dose levels to be tested were not indicated in the protocol. Note that the highest dose level tested should result in definite pharmacological signs. The dose levels which resulted in signs of kidney pathological changes may be used as a guide, but since the method of generation for each study is different, the blood levels are a more important index of exposure."

Response: We agree that the dose levels tested should produce pharmacological signs. However, as discussed above, these doses might not produce blood levels that show a dose-related increase in lindane and are in the same range as those in the rat study. Thus, the EPA needs to decide which is the more critical criterion for selecting exposure concentrations, i.e. toxicity or blood levels.

Ann M. Blacker

Ann M. Blacker, Ph.D.
Toxicologist
March 12, 1987

Glenn S. Simon

Glenn S. Simon, Ph.D., D.A.B.T.
Manager of Toxicology
March 12, 1987