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

JUN 30 1989

007304

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No.: 52904-C. Lindane: Review of a fourteen week dust inhalation study with mice.

TOX CHEM No.: 527
TOX PROJECT No.: 9-0301
Record No.: 234362

FROM: John Doherty 5/24/89
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

TO: George LaRocca
Product Manager #15
Registration Division (H7505C) 5/24/89

THROUGH: Robert Zendzian
Acting Section Head
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

THROUGH: Edwin Budd
Acting Branch Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C) 6/26/89

The Centre International d'Etudes du Lindane (C.I.E.L.) has submitted a fourteen week inhalation toxicity study with lindane in mice in response to a previous requirement from the Agency (refer to the letter from the law office of McKenna, Conner and Cuneo representing the C.I.E.L. and dated November 2, 1988).

Toxicology Branch I (TB-I) has reviewed the study and the following comments apply.

1 of 28

Toxicology Branch I Comments

1. This study has been reviewed and determined to be CORE GUIDELINE.

The conclusions for this study are that the NOEL is 0.3 mg/m^3 (0.3 ug/l) and at the LEL of 1.0 mg/m^3 there are increased deaths due to lindane exposure in both male and female mice. The high dose group females had a 14% increase in liver weight.

2. There were no effects of lindane on the kidneys of the exposed mice as indicated by either urinalysis (functional effects) or pathology. Note: There were special aspects of the study included to assess for possible adverse kidney effects because the kidney is regarded as a possible target organ for toxicity.

3. There were no obvious effects of lindane on the formed elements of the blood in the exposed mice as indicated by extensive myelograms.

Studies Reviewed

1. Subchronic Inhalation study in mice-5 day range finding. Bushy Run Research Center, #50-595, April 15, 1988.	No deaths and only "equivocal" evidence of lindane toxicity. SUPPLEMENTARY Doses tested: 0, 1 and 10 mg/m^3.
2. Subchronic Inhalation study in mice-14 weeks. Bushy Run Research Center, #51-524, October 7, 1988.	GUIDELINE NOEL = 0.3 mg/m^3. LEL = 1.0 mg/m^3, deaths in both males and females. At 5-10 mg/m^3 more deaths. Increased female liver weight (14%). Doses tested: 0, 0.3, 1.0 and 5-10 mg/m^3.

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Reviewed By: J.D. Doherty 6/13/89
Section I, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Robert Zendzian
Section I, Toxicology Branch I - IRS (TS-769C) 6/13/89

DATA EVALUATION REPORT

Study Type: 82-4 - Subchronic Inhalation - Mice

MRID No.: 408735-01 (2 Volumes) TOX Chem No.: 527

Test Material: Lindane Technical, ID No. E-2722, Batch #DA433 from Rhone-Poulenc Agrochemie, Lyon, France

Synonyms: Hexachlorocyclohexane (gamma-isomer)

Study Numbers: BRRC #51-524 and Metpath #14014.

Sponsor: Central International D'Etude Du Lindane (CIEL)

Testing Facility: Bushy Run Research Center, Export, PA

Title of Report:

Lindane Technical Fourteen-Week Dust Aerosol Inhalation Study on Mice.

Authors: D.R. Klonne and W.J. Kintigh

Report Issued: October 7, 1988

Conclusions:

NOEL = 0.3 mg/m^3; LEL = 1.0 mg/m^3, at this level there were two unexplained deaths and at 5 to 10 mg/m^3 there were 20 (5 male and 15 female) deaths. The cause of death was not determined. No effects noted on kidney function, pathology or in bone marrow. Liver weight in the high dose (5 mg/m^3) group females was elevated 14%.

Classification: GUIDELINE

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

A statement signed by Linda [illegible]. Caliste, Group Leader, Good Laboratory Practices, Quality Assurance attested that numerous (about 16) inspections were made during the course of the study.

REVIEW

In this study four groups of 45 mice (CD-I strain, from Charles River, Kingston, NY) per sex per dose group were dosed with either 0, 0.3, 1.0, or 5 to 10 mg/m^3 of lindane generated into the atmosphere by Wright Dust Generators. Each group of 45 mice of each sex consisted of subgroups of 15 mice/sex which were scheduled to be sacrificed after 7, 14, or 20 weeks. The group sacrificed at 20 weeks was a recovery group which was not exposed to lindane after the 14th week. Of the 15 mice in each subset, 10 were scheduled for pathological evaluation and the remaining 5 were scheduled for serum lindane concentration determinations. The control group was exposed to air only.

The test material used was obtained from Rhone-Poulenc Agrochemie, Lyon, France and was from Batch #DA433. Analysis of this material indicated that it was 99.6% gamma isomer.

The aerosol was generated using Wright Dust Generators. A model "Wright Dust Feed II" was used to generate the atmosphere for the 1 and 5 to 10 mg/m^3 atmospheric levels and a model "Wright Dust Feed" was used to generate the atmospheric level of 0.3 mg/m^3. The atmospheric lindane coming through the Wright Dust Generator was passed through a glass elutriation chamber prior to entry into the exposure chamber so that larger particles were removed resulting in a reduction of particle size distribution. The report states that the dust was dispersed throughout the chamber by chamber air supply and a "dispersion device operated with compressed air (10 psig)." This "dispersion device" was not further described.

The test dose levels were selected based on a preliminary range-finding study (Appendix 12, Bushy Run Research Center Study No. 50-595 dated April 15, 1988, DER attached) which showed that <u>males</u> did not develop definite toxic signs after exposure to lindane at 1.0 and 10.0 mg/m^3 for 6 hours per day for 5 daily exposures using similar exposure conditions. Females were not assessed.

RESULTS

1. <u>Chamber concentrations of Lindane and Particle Size Distribution</u> - Atmospheric concentration - Two samples, each reported as being of 150 minutes' duration, were said to be taken from each study chamber each day and gravimetric analysis of the trapped material was made. The gravimetric analysis showed that the chamber concentrations were 0.25 ± 0.062, 1.04 ± 0.146, and 4.94 ± 1.477 mg/m^3 of test material for the low- (0.3), mid- (1.0), and high- (5.0) dose test groups. During the first five exposures, the high dose group was exposed

to a mean daily concentration of 9.72 ± 0.49 mg/m^3. There was no evidence that this group was exposed to an unusually high atmospheric concentration (other than the stated level) during the period when most of the females died (see below).

The mass median aerodynamic diameter in microns for the atmospheric particles was 3.1 to 3.3 with geometric standard deviations of 1.7 to 1.8. [Note: No particle size determinations were made on days 17 to 40 because the apparatus used to assess particle size malfunctioned and required repair during that period.]

2. Behavioral Reactions and Mortality - The study report states that "There were no exposure-related clinical signs observed during the study."

In spite of the fact that no clinical reactions were noted, there were many deaths among the mice dosed with 10 mg/m^3 during the first week of the study. After lowering the high-dose level from 10 mg/m^3 to 5 mg/m^3 there were fewer deaths noted. The mice which died, died overnight, and no notice of the symptoms preceding death were made.

The following table illustrates the frequency of death in this study.

	Deaths		
Group	Males	Females	Total
Control	0	2	2[1/]
0.3 mg/m^3	0	0	0
1.0 mg/m^3	1	1	2
5.0 mg/m^3	5 (2)	15 (12)	20 (14)[2/]

1/One of the control mice (a female), died as a result of a "caging accident." The other was described in this report only as "found dead."
2/Two of the males and 12 of the females died when their dose level group was exposed to 10 mg/m^3. All other mice died when the chamber atmospheres were as shown.

The single male in the mid-dose group which died, died during the seventh week of exposure. The single female in the mid-dose group which died, died during the ninth week. No cause of death was determined for either mouse.
Of the 3 of the 5 remaining high dose group males which

died, two died during the recovery phase (nonexposed), the third died on day 9 of the study.

Of the three females not dying during the first week, 2 deaths were on day 22 and the third was on day 64.

The study report concludes that the NOEL for deaths is 0.3 mg/m^3, the low dose level "because two deaths did occur in the 1 mg/m^3 group that cannot be explained or discounted."

3. Ophthalmic Evaluations - Ophthalmic evaluations were made at pretest, at weeks 7, 14, and after the recovery phase following dilation with 1% Mydriacyl. No exposure-related eye lesions were reported.

4. Body Weights, Food, and Water Consumption - No consistent effects on body weight or body weight gain were noted in this study. Body weights were determined on a daily basis.

   No effects on food or water consumption were reported.

5. Hematology and Clinical Chemistry - Samples were taken at 7th, 14th, and 20th weeks. When available 10 mice/sex/group were evaluated. The blood was drawn from the orbital sinus of methoxyflurane-anesthetized mice prior to sacrifice.

   The following hematologic parameters were reported as being measured or calculated: leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. Blood smears for differential leukocyte counts were prepared and evaluated for all animals of the control and high concentration groups. (Leukocyte smears were also evaluated for the low and intermediate concentration groups at Week 7 - protocol deviation.) Reticulocyte smears were also made.

   No consistent dose-related effects of exposure to lindane were reported on any of these parameters.

   The following clinical chemistry analyses were performed:

   a. Glucose
   b. Urea nitrogen (UN)
   c. Creatinine
   d. Aspartate aminotransferase (AST)

e. Alanine aminotransferase (ALT
f. Total protein
g. Albumin
h. Globulin
i. Total bilirubin
j. Direct bilirubin
k. Indirect bilirubin
l. Gamma-glutamyl transferase (GGT)
m. Sorbitol dehydrogenase (SDH)
n. Alkaline phosphatase (ALK)
o. Calcium
p. Phosphorus
q. Sodium
r. Potassium
s. Chloride
t. Carbon dioxide (CO_2)

No consistent effects of lindane exposure on any of these parameters were reported as evident. Some changes in K^+, globulin value, glucose and blood urea nitrogen were noted but not considered to be related to lindane exposure since there was no dose dependence or consistency at 7 and 14 weeks. The blood urea nitrogen increase in the mid- (33%) and high- (44%) dose females at 20 weeks was considered as a possible kidney effect but this was not supported by other observations in the females such as pathological changes.

6. Urinalysis - [Note: Since the kidney is regarded as a target organ for lindane, a special water loading procedure was utilized for this study.]

Urinalysis was assessed at weeks 7, 14, and 20 using the same mice that were used for hematology and clinical chemistry. They were dosed by gavage (30 mL/kg) with tap water just before being placed in metabolism cages. The urine was collected over a 14- to 15-hour period and assessed for the following: volume, color, turbidity, creatinine, sodium, potassium, chloride, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, microscopic constituents, and osmolarity. The methods used for each parameter were listed.

No consistent dose-related effects of exposure to lindane were evident in either sex in these urinalysis assessments. There were several deviations from the normal which were noted but dismissed by the testing laboratory as not being related to lindane exposure. These are listed as follows:

- Blood in urine in the low-dose groups at 20 weeks (not in other groups at higher dose levels or during the actual exposure period).

- Urobilinogen in female high-dose group at 20 weeks (not present during exposure period and no histological changes in the liver).

- Creatinine decreased at all exposure levels at 7 weeks (no dose response).

-potassium decreased in the male mid- and high-dose groups at 7 weeks (not at other times in males).- Potassium decreased in the female high-dose group (statistically significant) but in mid- and low-dose groups it was not significant but still higher. (This change was the opposite as that seen in males at week 7, was not observed during actual exposure to lindane, and there was no evidence of supporting histopathology.)

7. Bone Marrow Analysis - [Note: In a previous review of a rat subchronic inhalation study (refer to J. Doherty review dated April 25, 1986 for EPA Special Review of Lindane), the bone marrow was implicated as a possible target organ for lindane as indicated by changes in the composition of the elements in the marrow. Thus, bone marrow analysis was specifically requested for this study.]

Bone marrow samples were reportedly taken from the sternum and femur (right) from 10 mice of each sex at each sacrifice interval. Only the smears from 10 (when available) mice per sex from the controls and high-dose test groups were evaluated microscopically. Preparation of the bone marrow smears consisted of staining with "Pappenheim Panoptic Method" at the BRRC but were read microscopically at MetPath Laboratory in Rockville, Maryland. A separate report, Appendix 5, discussed the results of the bone marrow analysis and was signed by Edward H. Fowler, D.V.M. Dr. Fowler's conclusion was that although some statistically significant differences between the control and high-dose groups were evident, there was no consistent change in any particular cell type(s). Thus, there was no change which he considered an effect due to lindane exposure. [Note: The actual reading of the bone marrow slides was by Drs. Walter F. Loeb and Gregory S. Travlos. 200 cells from each smear were reported as usually being counted.] Thirty-seven different parameters were investigated and compared statistically

for the control and high-dose group for both sexes at three sacrifice intervals. A summary of the statistically significant increases/decreases as determined by the pathologist is attached. This table shows that there was no consistency over time for the change of given parameters, that the sternum and femur often showed the opposite effect and there were no correlations between the changes noted in males and females.

Toxicology Branch I (TB-I) concurs that the study does not demonstrate an effect of lindane on blood elements in bone marrow in mice at the exposure level of 5 mg/m^3.

8. Organ Weights - The brain, liver, kidneys, lungs, spleen, thymus and adrenal glands, and testes (from the males) were weighed at the 7, 14, and 20 weeks sacrifice intervals. Absolute, organ to body weights and organ to brain weights were tabulated and assessed.

Inspection of the data submitted indicated that there were no consistent or dose-related differences in these organ weights. In particular, the liver (except for females at week 20), kidneys, and spleen, all of which might be considered as target organs for effects of lindane did not show statistically significant increases. The liver weight increase for females at week 20 (+14%, statistically significant $p < 0.05$) is considered spurious by the testing laboratory and there was no evidence of associated pathology.

No evidence of lung weight increases indicative of edema formation were evident in the interim, terminal or recovery sacrifice groups. Lung weight for the decedents was not obtained. There would not be appropriate control group animals to compare the decedents lung weight with.

9. Necropsy - No evidence of increased incidence of grossly observable lesions were evident. Necropsy was performed on all mice in each group.

10. Histopathology - The 14-week group sacrifice was subjected to the most extensive histopathological examination. For instance, some 37 tissues were assessed for all available control and high-dose mice at week 14. At weeks 7 and 20, only seven tissue/organ types (including the kidney) were assessed microscopically.

No special staining techniques were used and apparently

only one slide of the kidneys was prepared and read. The mice reserved for serum lindane concentration at each sacrifice interval were also assessed microscopically for kidney lesions. Overall the kidney tissue from 45, 25, 25 and 45 male and female mice from each dosage group were reported as being examined microscopically. The kidney tissue from the 10 mice scheduled for sacrifice at weeks 7 and 20 were not assessed microscopically. The kidney tissue from the mice which died during the course of the study was evaluated macroscopically.

Inspection of the kidney data indicates that although several lesion types were present, they were not present in a dose-dependent manner or include the tubular swelling or degeneration with necrosis or an increase in hyaline droplet formation which was noted in other studies with lindane particularly in male rats.

The study report maintains that there were no consistent dose-related lesions in any organ to indicate an effect of lindane exposure.

TB-I notes, however, that at 14 weeks there was an increase (statistically significant, $p < 0.05$) in "spindle cell hyperplasia" in the adrenals in the high dose female group. Five of the seven mice in the high dose female group (71.4%) but only one of ten mice in the control group (10%) had this condition. The mice in the low and mid dose group were not examined at 14 weeks and the adrenals were not examined at weeks 7 and 20 for any mice. Among the males, at 14 weeks only 2 of 10 control mice and none of ten high dose group mice had this condition in the adrenals.

These data suggest a possible effect on lindane on the adrenals as indicated by increased incidence of "spindle cell hyperplasia" in females. This suggestion of a possible effect was further evaluated by Dr. Leonard Slaughter (TB-I consulting pathologist). Dr. Slaughter telephoned Dr. Ann Blacker of the Rhone-Poulenc Co. and requested certain additional information (refer to copy of letter from J. Doherty to Dr. Blacker dated May 4, 1989, attached). In response, the C.I.E.L. (refer to letter from Charles A. O'Connor, III dated June 1, 1989, attached) provided a more complete description of the condition "spindle cell hyperplasia" and comments on its spontaneous occurrence in the CD-1 strain of mouse. Sufficient information was provided to allow Dr. Slaughter to conclude that the apparent increase in the incidence in "spindle cell hyperplasia" is within expected spontaneous natural occurrence and

not convincingly related to lindane exposure.

Inspection of the data for the mice which died during the study (decedents) did not provide indications as to how the mice died. For example, the high dose group males (3/5) and high dose females (11/15) and controls (2/2) had "color change, focal/multifocal" as a gross necropsy observation in the lungs. Microscopically, 3 of 5 males in the high dose group were reported to have "congestion" as well as singular incidence of six other generalized lesions in the lungs. Among the females, microscopy revealed that 2/2 controls and 10 of 15 high dose group females had "congestion" and 2/2 controls and 5/15 high dose group females had "hemorrhage". There were no indications based on microscopic assessment of the lung that the mice died of an edema resulting from exposure to atmospheric lindane.

Note: The pathology report was prepared and signed by P.E. Losco D.V.M., and is presented in Appendix 7.

11. Serum Lindane Concentration

Serum lindane concentrations were determined at weeks 7, 14, and 20 for both males and females. Five males per sex were scheduled for assessment but were not always available. The report is in Appendix G and is signed by M.A. Vrbanic and J.P. Van Miller. The serum was analyzed for lindane by gas-chromatography. The sample was reportedly taken at the end of the daily exposure.

The following table illustrates the findings.

	Lindane (in ppb)			
	7th Week		14th Week	
	M	F	M	F
Control	ND	ND	ND	ND
0.3 mg/m^3	32 ± 16	94 ± 31	22 ± 7	41 ± 22
1.0 mg/m^3	71 ± 36	124 ± 30	52 ± 23	85 ± 38
5.0 mg/m^3	87 ± 21	179 ± 108	152 ± 55	290 ± 27

ND means less than the limits of detection at 1 ppb.

Serum levels in lindane at week 20 were below the limits of detection.

This table indicates that the females have higher levels of lindane in the serum than males.

No tissue levels of lindane were determined.

Conclusion

This study is classified as CORE GUIDELINE.

The following NOEL and LEL are assigned for this study.

NOEL = 0.3 mg/m^3.

LEL = 1.0 mg/m^3. At this level there were 2 unexplained deaths (one male and one female). At 5-10 mg/m^3, there were 20 deaths (5 male and 15 female). The cause of death was not determined. No signs of toxicity were observed and the deaths occurred at night.

At 5 mg/m^3, liver weight in the high dose group females was elevated 14%.

No effects were noted on kidney function or structure or in the bone marrow.

Lindane toxicology review

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Pages 14 through 15 are not included in this copy.

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Reviewed by: John Doherty John Doherty 3/1/89
Section I, Toxicology Branch I, (TS-769C)
Secondary reviewer: Edwin Budd
Section I, Toxicology Branch I, (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic inhalation - mice (Pilot Study)

ACC. NO.: 408735-01 (Volume 2, Apppendix 12)

TOX. CHEM. NO.: 527

TEST MATERIAL: Lindane Technical (99.5% pure) obtained from the Rhone-Poulenc Co. from Batch DA 433)

SYNONYMS: hexachlorocyclohexane (gamma isomer)

STUDY NUMBER(S): 50-595

SPONSOR: C.I.E.L.

TESTING FACILITY: Bushy Run Research Center

TITLE OF REPORT: "Lindane Technical: Five-day Inhalation Pilot Study on Mice".

AUTHOR(S): D.R. Klonne and W.J. Kintigh

REPORT ISSUED: April 15, 1988

CONCLUSIONS:

Male mice exposed to 0, 1 and 10.0 mg/m^3 showed increases in lindane concentraions in the blood but only "equivocal evidence of toxicity. Females were not tested.

Classification: CORE-SUPPLEMENTARY

Special Review Criteria (40 CFR 154.7) N/A

Quality Assurance Statement:

A statement signed by Linda J. Calisti attested that as many as 9 Quality Assurance inspections were made including inspections of the protocol, in-life phase, clinical pathology and analytical aspects and of the archives.

Review

[Note not reviewed in critical detail.]

A copy of the study report summary is attached.

Lindane toxicology review

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

May 4, 1989

Dr. Ann M. Blacker
Toxicologist
Rhone Poulenc Agrochemical Division
P.O. Box 125 Monmouth Junction
New Jersey 08852

Dear Dr. Blacker:

This letter is in response to your request to Dr. Leonard Slaughter to provide in writing the content of his telephone request seeking certain additional information regarding the 14 week inhalation toxicity study in mice with lindane (Bushy Run Research Center, Report #51-524).

The following information should be provided:

1. A written description of the spindle cell hyperplasia in the adrenal gland.

2. Historical control data on the adrenal gland from the testing laboratory's experience.

3. State when we (EPA) can expect to receive your written description of the spindle cell hyperplasia and the historical control data as above.

Sending this information directly to me will expedite completion of the review of the 14 week inhalation study. I would also advise you to send a copy to the product manager to assure that the submission is properly logged in the Agency's files.

RECEIVED
1 0 1989
ANN M. BLACKER

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Thank you for your attention to this matter.

Sincerely,

John D. Doherty, Ph.D., D.A.B.T.
Health Effects Division (H7509C)
Environmental Protection Agency
401 M St. SW
Washington, D.C.
20460

cc: Dr. Leonard Slaughter
Mr. George LaRocca
Dr. Dana Pilitt

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June 1, 1989

Mr. George LaRocca
Product Manager, Team 15
U.S. Environmental Protection Agency
Crystal Mall, Bldg. No. 2, Room 204
Arlington, VA 22202

Re: CIEL/Lindane -- Submission of Requested Additional Information Regarding the 14-Week Inhalation Study on Mice with Lindane

Dear Mr. LaRocca:

On behalf of our client, Centre International d'Etudes du Lindane ("CIEL") and its three members holding U.S. registrations for the insecticide lindane, Rhone-Poulenc Ag Company (representing Rhone-Poulenc Agrochemie), E.M. Industries, Inc. (representing Shell Agrar GmbH & Co., KG), and Inquinosa (Industrias Quimicas del Noroeste, S.A.), we are herewith submitting the additional information requested in a May 4, 1989 letter from Dr. John D. Doherty regarding the 14-week inhalation study in mice with lindane (Bushy Run Research Center, Report # 51-524).

Dr. Doherty's May 4, 1989 letter (which formalized an earlier telephonic request by Dr. Leonard Slaughter) requested the submission of a written description of spindle cell hyperplasia in the adrenal gland, and the testing laboratory's historical control data on the adrenal gland. In response to this request, we have enclosed herewith a copy of Dr. Doherty's letter along with the following three (3) documents:

(1) An April 25, 1989 letter from P.E. Losco, VMD of the Bushy Run Research Center ("BRRC") which describes in detail the spindle cell hyperplasia observed in the study in question, including the frequency, location and severity of the

discovered instances. In summary, it has been found that the presence of a lesion related to spindle cell hyperplasia is of no biological significance, in that it has not had any impact upon the functioning or life expectancies of the studied mice, and has not led to the progression of other diseases. This letter additionally explains that in a recent chronic study using CD-1 mice from the same source as the lindane study, 18 of 19 control group females had the lesion at the 12-month sacrifice. It further notes that the incidence of the lesion is close to one hundred percent (100%) in lifetime studies using the CD-1 strain. Spindle cell hyperplasia does not cause early death, as the animals have lived full term despite the presence of the lesion. Although the incidence of the lesion in the 14-week lindane study was higher than ordinarily expected in younger animals, the BRRC study director concludes that the somewhat elevated incidence in treated mice is due to random biological variation, since it still falls within the normal range of variation for a 14-week study of this type.

(2) A Summary of Microscopic Diagnoses for the study in question, which includes data regarding the occurrence, frequency, and severity of the spindle cell hyperplasia in the studied animals, as well as a notation of an observed frequency variance from a control group.

(3) A copy of an article by Dawn G. Goodman, which has been excerpted from a monograph on pathology regarding the endocrine systems of laboratory animals. The article describes, in great detail, the appearance, microscopic features, ultrastructure, differential diagnosis, and biological features of spindle cell hyperplasia in the adrenal gland of the mouse. The article also includes a brief comparison with spindle cell hyperplasia in other species.

We believe that these documents respond in full to the requests in Dr. Doherty's May 4, 1989 letter.

In addition, please note that CIEL expects to submit the addendum on the bone marrow results from the 14-week study by June 9, 1989.

If you have any questions or comments concerning this submission, please contact me at the above number or my associate, Gordon Richman, at (202) 789-7572.

Sincerely,

McKENNA, CONNER & CUNEO

Charles A. O'Connor, III
Counsel for CIEL

Enclosures

CAO/amh
cc: Dana Pilitt
Dr. John Doherty

Lindane toxicology review

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LaVia MF, Hill RB Jr (1975) Principles of pathobiology, 2nd edn. Oxford University Press, New York, p 53
Robbins SL, Cotran RS (1974) Pathologic basis of disease. Saunders, Philadelphia, p 47
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Subcapsular-Cell Hyperplasia, Adrenal, Mouse

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Synonyms. Subcapsular-cell reaction; spindle-cell hyperplasia of the adrenal cortex; type A cell hyperplasia; fibrous degeneration of the zona glomerulosa; cortical scars.

Gross Appearance

Generally, this lesion is not observed grossly. In older mice, the capsular surface of the adrenal may be slightly irregular. Occasionally, nodules may be present if the lesion has become focally extensive or developed into a neoplasm (p. 50).

Microscopic Features

In older mice of many strains proliferation of spindle cells, either focally or diffuse, occurs beneath the capsule of the adrenal gland (Fig. 62). This change may involve the zona glomerulosa and extend downward into the cortex between the cords of the zona fasciculata, often having a wedge shape. It may be so extensive as to replace large portions of the cortex. Occasionally, the proliferation may be nodular and may progress to neoplasms.

Microscopically, the cells are oval to fusiform with elliptical nuclei and scant, basophilic cytoplasm (Fig. 63); mitotic figures are rare. These

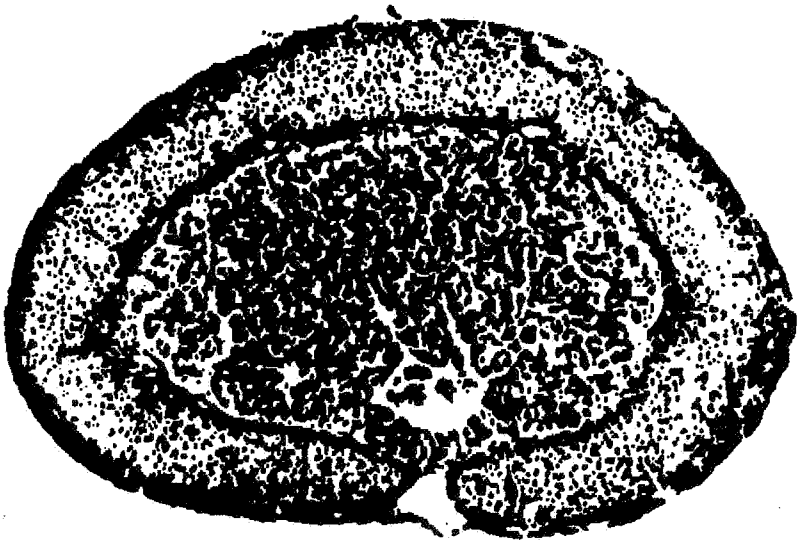

Fig. 62. Diffuse subcapsular-cell hyperplasia of the mouse adrenal gland with foci extending downward into the cortex. H and E, ×65

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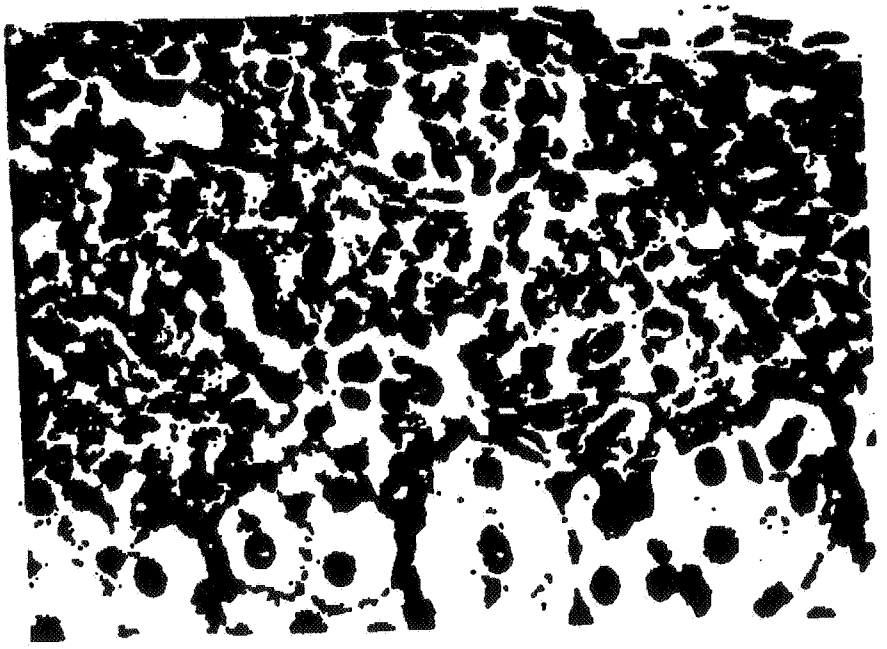

Fig. 63. Subcapsular-cell hyperplasia, composed of oval to fusiform type A cells. H and E, ×900

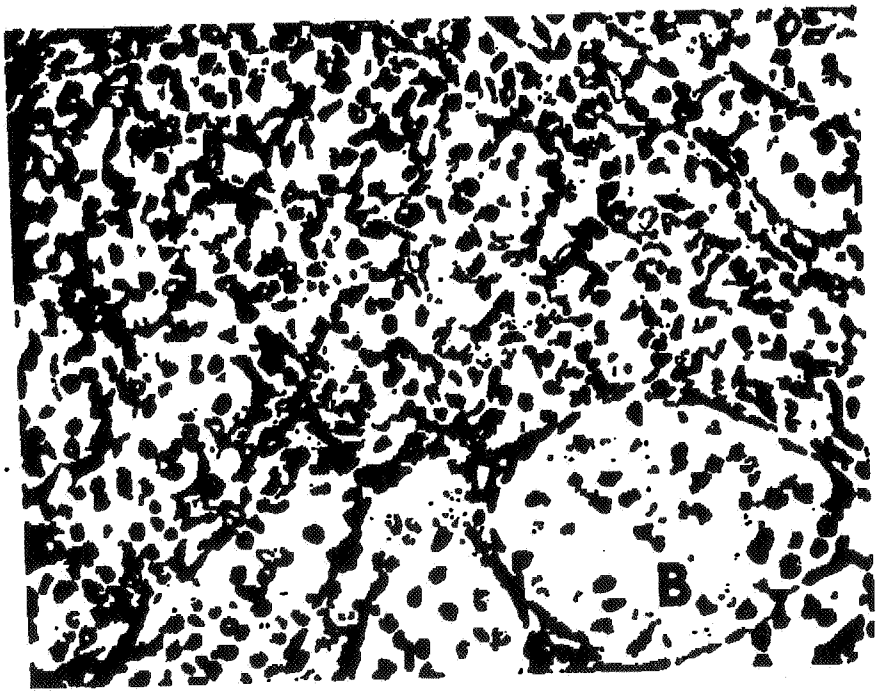

Fig. 64. Subcapsular-cell hyperplasia, composed primarily of nests of large, polygonal type B cells *(B)* intermixed with fusiform type A cells *(A)*. H and E, ×330

cells have been referred to as type A cells and are the predominant cell type seen. Type B cells are occasionally observed, usually when the lesion is extensive. However, they are most commonly found in neoplasms. They are large, round to polygonal cells with abundant eosinophilic or vacuolated cytoplasm and round, vesicular nuclei. They may occur as single cells scattered among the spindle cells or in small nests surrounded by the spindle cells (Fig. 64).

Ultrastructure

Sato (1967) studied the fine structure of the mouse adrenal cortex at various ages. He described thickening of the capsule with age, associated with an increased number of cell layers. With electron microscopy an increase in collagen fibrils and in the number of capsular cells was seen. Particularly in older animals capsular cells contained few organelles, consisting primarily of polygonal

mitochondria, occasional lipid droplets, and membrane-bound dense bodies. Desmosomes were observed attaching adjacent capsular cells and membranes surrounding groups of capsular cells resembled basement membranes. In Sato's opinion, these cells may represent reserve cells of the zona glomerulosa."

Differential Diagnosis

This lesion is quite characteristic microscopically. In the past it has been thought to represent scarring (Löwenthal 1931; Whitehead 1932) or "fibrous degeneration" of the zona glomerulosa (Delost et al. 1958). The primary problem in diagnosis arises with extensive focal lesions i. e., whether the lesion is a focal hyperplasia or has progressed to neoplasia. When the proliferation is large, nodular, and compresses adjacent tissue, it is generally considered to be neoplastic. (p. 50).

Biological Features

Subcapsular-cell hyperplasia is commonly observed in older mice of many strains. The incidence of the lesion in various strains is not well documented. It is rarely observed in young mice, but is more frequent and more severe with age. In some strains, the lesion tends to be more extensive in females than in males. The severity of the lesion also varies greatly between individuals of the same strain, sex and age.

Gonadectomy usually enhances its development in both male and female mice. The lesion develops at an early age following castration, becoming quite severe, and adrenal cortical tumors usually arise from the lesion in such animals (Dunn 1970, 1979; Woolley 1950).

Comparison with Other Species

Subcapsular-cell hyperplasia is rarely seen spontaneously in other species. Nodular subcapsular spindle-cell proliferations, including neoplasms, have been described as occurring spontaneously in hamsters (Pour et al. 1976), increasing in size after castration (Cantin 1971; Murthy and Russfield 1966; Russfield 1966). Subcapsular-cell proliferations in rats (Cardeza 1956) and nodular spindle-cell lesions in goats (Richter 1958) have been described as observed following gonadectomy.

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