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OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

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MEMORANDUMSUBJECT: Lindane - Special Review: Review of a Rat Subchronic  
Inhalation Toxicity Study with LindaneTox. Chem. No. 527  
Project No. 1232  
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and

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Toxicology Branch  
Hazard Evaluation Division (TS-769C)Background:

The Centre International d' Etudes du Lindane (C.I.E.L.) has submitted a 90-day subchronic inhalation toxicity study with the insecticide lindane. This study was originally

*Added  
4/23/86*

submitted as written in German and EPA subsequently requested that the study be translated into English before Toxicology Branch (TB) would review it. The English translation of the study has been reviewed (see below).

The 90-day inhalation study with lindane was submitted in response to EPA's Data Call-In Notice which requested submission of this study in support of certain uses of lindane including subterranean termite control. The C.I.E.L. asserts that the test data show no effects due to exposure to lindane except for reversible clinical symptoms and increased concentrations of cytochrome P-450 in the liver of the rats exposed to the highest concentration tested.

#### Toxicology Branch Conclusions

1. Toxicology Branch (TB) does not concur with the conclusions made in the study report and has determined that there are at least two other effects of exposure to lindane than the report recognizes. These are:

- a. Transient histopathological and weight changes in the kidney of male rats at the two highest test dose levels.

TB is especially concerned with this observation because the kidney of male rats has already been identified as a target organ for lindane in a rat 90 day feeding study (refer to the Registration Standard for lindane and the original review by Dr. K. Locke dated June 17, 1983).

In particular, TB has noted that the male rats have increased occurrences of lesions described as "cloudy swelling of the tubule epithelia" in the two highest test dose groups. In addition, two types of lesions were noted in the two highest test groups that were not found in the control or lower dose test groups. These were lesions described as "dilated renal tubules with protein containing contents" and "proliferated tubules".

A NOEL for kidney weight changes and histopathological changes is set at 0.1 mg/m<sup>3</sup>.

- b. Changes in the composition of the cellular components of the bone marrow as evidenced by the myelograms of this tissue.

The pattern of changes noted in the bone marrow myelograms indicated that exposure to 5 mg/m<sup>3</sup> results in stimulation of the formation of blood cells although other factors may be involved. Since only the high dose test group and the controls were assessed, no NOEL or LOEL could be established for this alleged effect of lindane.

The registrant should be asked to present their defense (if they wish) that the qualitative changes noted (see page 8 of this review) are not the result of exposure to lindane.

Based on the observations in this study regarding the possibility that lindane may affect the dynamics of blood cell formation, the composition of the bone marrow (myelograms) must be determined for all future subchronic and chronic studies with lindane.

2. The study is classified as CORE MINIMUM.
3. Other effects observed in this study are summarized on page 15.

Study Type: Inhalation - subchronic [90-day inhalation study on rats with lindane].

Accession No.: 255003

MRID No: -

Sponsor: Centre International d'Etudes du Lindane (C.I.E.L.).

Laboratory: Fraunhofer Institut für Toxikologie und Aerosolforschung (D-5948 Schmollenberg, Germany)  
Project No. 104264

Date: February 28, 1983 (as originally reported and written in the German language).

Approximate date of translation - December 1985.

[Note: The study report as reviewed herein is an English translation of the original German language report. The name of the translator and his/her credentials were not provided in the document. The translator did not provide a signed statement qualifying that the English version represents an accurate translation of the German document.]

B. The test material used for this study was lindane and was identified as being from batch number 79044/174 and of 99.9 percent purity and described as being a white powder.

#### Generation of the Aerosol and Exposure Conditions

Lindane was generated into an aerosol by means of a La Mer generator. The principle of this generator system consists of first nebulizing a solution of NaCl into a cyclone chamber such that only small droplets of saline solution escape the chamber and the water evaporates leaving dry particles of sodium chloride which in turn form a condensation nuclei for the vaporized test material. The test material (which must be stable to heat) is separately heated to vaporize and the resulting vapor is mixed with the air stream containing the dry particles of sodium chloride. The air mixture of lindane (or other test material) is cooled and the lindane condenses on the particulate sodium chloride. In order to achieve desired atmospheric concentrations of the test material, the aerosol as generated is diluted by serially mixing with air before passage into the test chambers.

### Analyses of the Test Chambers for Atmospheric Concentrations of Lindane

Samples of the aerosol were trapped on membrane filters (0.2  $\mu$ m) which were located near both the inlet and outlet tubes. The atmosphere was also assessed for the "gas fraction in the aerosol" by utilizing gas wash bottles containing toluene. The samples were drawn through the membrane filters and gas wash bottles by means of a membrane pump and collection times were 13 minutes for the higher concentrations and 26 minutes for the lowest concentration at set withdrawal volumes.

The membrane filters were washed with toluene and quantitated for their lindane content.

The atmospheric concentrations for the exposed groups were reported to be as follows:

Group 1 (control)	0	mg/m <sup>3</sup>
Group 2	0.02	mg/m <sup>3</sup>
Group 3	0.12	mg/m <sup>3</sup>
Group 4	0.60	mg/m <sup>3</sup>
Group 5	4.54	mg/m <sup>3</sup>
Group 6* (control)	0	mg/m <sup>3</sup>
Group 7*	4.84	mg/m <sup>3</sup>
Group 8 (control)	0	mg/m <sup>3</sup>

\* Groups 6 and 7 were set aside for the recovery aspects of the study.

### Analyses of the Particle Size of the Aerosol

The particle size distribution was determined by means of an aerosol spiral centrifuge with oscillating quartz mass detectors. The results indicated that the aerosol had the following characteristics:

Arithmetic mean	1.11 $\mu$ m
Standard deviation	0.39 $\mu$ m
Geometric mean	1.03 $\mu$ m
Geometric standard deviation	1.45 $\mu$ m
Median	0.92 $\mu$ m

C. The test animals used in this study were male and female Wistar HAN/BOE, SPF rats. They were obtained from the Lippische Versuchstierjucht Company in Germany. At the initiation of exposure the males were 10 weeks old (200 to 230 grams in weight) and the females were 11 weeks old (160 to 170 grams in weight).

The basic experimental design consisted of 7 groups of 24 rats (12 males and 12 females) plus an extra control group of 10 rats of each sex. Thus there were five groups in the main study which consisted of exposure for 90 days and sacrifice after termination of exposure. Two groups were exposed for 90 days and allowed to recover for 6 weeks before sacrifice and assessment for effects. [Note: The report often confuses the recovery period and refers to the period being for 4 weeks. TB calculated the period to be 6 weeks based on the dates provided for starting and completion of the study.] The extra control group was included exclusively for the analysis and determination of cytochrome P450.

The experiment consisted of exposure of four groups to doses of lindane (0.02, 0.1, 0.5, and 5 mg/m<sup>3</sup>), nominal aerosol concentrations for 6 hours/day. The study protocol implies that the rats were exposed for 90 actual days during the experiment which lasted 113 days.

The rationale according to the study report for conducting a subchronic inhalation study with lindane relates to exposure to lindane via the atmosphere while lindane is being manufactured. No mention was made of the inhalation of lindane which also occurs as a result of the various applications of lindane in practical use.

Although a rationale was given for testing lindane by the inhalation route of exposure, no rationale was given for the selection of the test dose levels.

Data were presented on the content of lindane in the serum, liver, brain and fat from all rats. Exposure to even the lowest test dose level of lindane resulted in detection of lindane in these tissues. There were marked sex differences noted in the storage of lindane. The tissue levels detected in the exposed animals is described as follows:

1. Brain. Brain levels reached up to 5567 ng/gm in females and 2791 ng/gm in males in the highest test dose group.
2. Liver. The highest female dose group had a lower content of lindane (1151 ng/gm) than the mid dose female group (2374 ng/gm) and the differences were attributed to activation of the metabolizing system. The highest level among the males was 735 ng/gm in the high dose test group.
3. Fat. Fat accumulated by far the most lindane as lindane levels in this tissue reached 127,120 ug/gm for the high dose test group females and 58,260 ug/gm for the males.

The low dose group had levels of 2,110 ug/gm for the females and 0.888 ug/gm for the males.

4. Serum. The serum had the lowest level of detectable lindane being in the range of 388 (for males) and 274 (for females) ng/gm.
5. On recovery, there were still traces of lindane detectable in the tissues but most had been eliminated.

D. Survival. No rats died during the experimental exposure.

E. Clinical symptoms. The high-dose groups (nominal 5 mg/m<sup>3</sup>) in both the main experiment and the group set for the recovery phase were reported as having higher frequencies of diarrhea and piloerection, which were described as being "slight" but apparently all the rats (males and females) in these groups were affected. The results summary also states that "some females on 0.02 and 0.1 mg/m<sup>3</sup> had mild diarrhea for about 20 days after the first 2 weeks of the study, and also ruffed fur."

The study report asserts a NOEL of 0.5 mg/m<sup>3</sup> for this aspect of the study. This conclusion is supported by the reports of diarrhea and ruffed fur in the rats dosed with 5 mg/m<sup>3</sup> in both the main and recovery aspect (during dosing) of the study. [Note: The statement made in the results section referring to some rats being exposed to 0.02 and 0.1 mg/m<sup>3</sup> having these conditions was not supported by the data in the symptoms tables.]

F. No consistent dose-related exposure effects were evident on body weight gain (although the female group receiving 0.02 mg/m<sup>3</sup> weighed slightly more than the control group (+6%) at the end of 90 days of exposure for the main group only. There were no exposure-related effects on either food consumption or water consumption noted.

The NOEL for this aspect of the study is 5 mg/m<sup>3</sup>.

[Note: For sections G, H, and I below, hematology and clinical chemistry determinations were made at weeks -2, 0, +1, 6, 13, and 19 (recovery). Urinalysis was made at weeks 0 and 13 (no urinalysis was made for the recovery animals).

G. Hematology. The parameters investigated were erythrocytes, hemoglobin, hematocrit, MCH, MCHC, MCV, platelets, leukocytes, Hepato-Quids test and differential blood picture.

Although there were sporadic statistically significant differences in blood cell type counts noted, the data do not

indicate an obvious pattern of lindane-related effects. Neither the quantitative changes or their frequency indicated an effect of lindane.

[Note: No individual animal data were presented and the statistics presented by the study report could not be verified. Also, there were no data on the differential blood picture for males presented.]

Bone marrow myelograms were prepared by sampling the marrow and applying the Pappenheim staining technique and assessing the cell counts. The study results section reports that "evaluation of the myelograms recorded for both the male and female treated animals failed to reveal any differences from the controls in either the "90 day animal group" or the recovery animals".

TB has examined the data for the main "90 day animal group" assessed at the "13th" week and notes that several significant differences were evident. The data for the recovery animals was not found. The following table illustrates the percentage increases as determined by TB reviewer:

Cell Type	Males	Females
Reticulocytes	+108%	+55%
Stab (Stem) Cells	+31%	-5% (NS)
Myeloblasts	+33%	+8% (NS)
Promyelocytes	-27%	-29%
Myelocytes	-14%	-7% (NS)
Basophilic Erythroblasts	-10%	-8%
Polychromated "	-15%	+11% (NS)
Macrolymphocytes	+25%	+29%
Small and Medium Size Lymphocytes	-15% (NS)	-45%

(NS= not significant, others are statistically significant by 0.05 or better.)



These changes in the composition of the bone marrow have been interpreted by TB to indicate that exposure to lindane at 5 mg/m<sup>3</sup> impacts the dynamics of blood cell formation. In particular lindane appears to stimulate red blood cell formation and this is most evident by the increase in reticulocytes, myelolasts and stab (stem) cells in males. The fact that all three cell types are elevated lessens the likelihood that the high value obtained for reticulocytes (+108%) is a random variation. Females also appear to be affected. The changes in other cell types (lymphocytes) imply that other phenomena may be going on as a result of exposure to lindane. Although no changes were evident in the whole blood, the conditions of exposure in this study may have confined the observable difference to bone marrow only.

Since the rats in the lower exposure levels were not assessed for bone marrow effects, TB cannot determine if the numbers reported are natural variations to allow TB to concur with the study report.

These changes noted in the composition of the bone marrow are of concern to TB because of the alleged association between lindane and aplastic anemia. Although the changes noted in this species and at the concentrations tested do not provide an indication that an anemia would develop, at least an effect on the bone marrow is implied.

Since only the high dose test groups and the controls were assayed, no NOEL and LOEL can be assigned for this aspect of the study.

H. Clinical Chemistry - The following parameters were investigated: alkaline phosphatase, SGOT, SGPT, total bilirubin, total cholesterol, glucose, total protein, BUN, creatinine, AChE, ChE, Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>++</sup>. [Note: The determination of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>++</sup> content were made only at one occasion for the main group and for the recovery animals.]

There were no consistent dose-related changes in most of these parameters investigated to indicate a test chemical effect although there were many instances of statistically significant differences between the controls and the test groups. Measurements of GOT and glucose, in particular, showed many instances of differences when compared to the controls but no pattern of a test chemical effect was obvious.

BUN values particularly for the males at the 13th week for the main test were elevated for all groups but not for the groups set aside for recovery. Inspection of the data suggests that the control subgroup for the main group was low

relative to other times the control groups were assayed thus resulting in the appearance of an increase in the dosed groups. [Note: A change in BUN might be indicative of kidney damage.]

Serum  $K^+$  levels and  $Ca^{++}$  levels for males showed some indications of possible test chemical effects. Inspection of the data indicate that both control readings for  $K^+$  and  $Ca^{++}$  are low and the dosed values (except low-dose  $Ca^{++}$ ) cluster at a higher value. Thus, no definite effect of lindane can be concluded. This also appears true for the female  $Ca^{++}$  data.

The data for  $Na^+$  determinations show elevation for the lower dose test groups, but not the high-dose test groups (males or females). The differences do not indicate an obvious test chemical effect.

Because the test chemical lindane may affect kidney function and there were noted some histopathological changes in this organ as well as possibly also changes in kidney weights in this study (see below), data on the ionic composition of the blood are important in determining a functional change in the kidney. Because of the unexplained variations in the determinations as presented, the data with  $Na^+$ ,  $K^+$ , and  $Ca^{++}$  are disturbing in that these assays should have resulted in smaller standard deviations and more uniform values among the different groups in the study but no pattern of test chemical effects on these parameters is established. [Note: The 90 day oral dosing study with lindane did not show changes in the blood levels of these parameters but showed a definite degree of kidney pathology.]

Cytochrome  $P_{450}$  values were determined after 90 days of dosing and for the recovery phase of the study. The results are shown in the following table.

	Male	Female
Controls	0.720 <sup>1/</sup>	0.975
Controls (stall)	0.830	0.810
0.02 mg/m <sup>3</sup>	1.127	0.747
0.10 mg/m <sup>3</sup>	1.241	1.079
0.50 mg/m <sup>3</sup>	1.181	1.108
5.0 mg/m <sup>3</sup>	2.430 <sup>2/</sup>	1.694 <sup>2/</sup>
Recovery control	1.670	0.985
Recovery 5.0 mg/m <sup>3</sup>	1.420	1.075

<sup>1/</sup>The precise units were not given.

<sup>2/</sup>The high-dose group is considered to be meaningfully elevated due to the presence of the test material.

The individual determination values were not presented to conduct an independent statistical assessment.

The NOEL for this aspect of the study (clinical chemistries and including cytochrome P<sub>450</sub> determinations) is set at 0.50 mg/m<sup>3</sup>. The only parameter considered to be meaningfully affected by inhalation exposure to lindane is cytochrome P<sub>450</sub>.

I. Urinalysis: The parameters investigated included: nitrite, pH, protein, glucose, ketone, urobilinogen, bilirubin, blood, osmotic pressure, and sediment analysis. Only data for the 90-day analysis were presented and only group 1 (control) and group 5 (high-dose group) were assessed.

No qualitative differences in the control or high-dose test group was reported.

No specific kidney function tests were included.

J. Organ weights. Absolute and relative organ weights were determined for the brain, heart, lungs, liver, spleen, kidney (left and right), adrenal (left and right), hypophysis, thymus, salivary gland (left and right), thyroid (left and right), prostate (ventral) testicle (left and right) or ovaries (left and right) for both the main group and recovery-phase rats.

#### 1. Kidney

	Absolute Weight (gm)		Relative Weight	
	Right	Left	Right	Left
<u>Males</u>				
Control	1.030	1.015	0.267	0.263
0.02 mg/m <sup>3</sup>	1.033	1.016	0.256	0.252
0.10 mg/m <sup>3</sup>	0.982 (-4.7%)	0.948 (-6.7%)	0.272	0.252
0.50 mg/m <sup>3</sup>	1.131 (+9.8%)	1.097 (+8.0%)	0.289 (+8.2%)*	0.280 (+6.9)
5.00 mg/m <sup>3</sup>	1.151 (+11.7%)*	1.094 (+7.8%)	0.318 (+19.1%)*	0.303 (+19.2)**
<u>Females</u>				
Control	0.607	0.578	0.267	0.255
0.02 mg/m <sup>3</sup>	0.643 (+5.9)	0.616 (+6.6)	0.267	0.256
0.10 mg/m <sup>3</sup>	0.640 (+5.4)	0.614 (+6.2)	0.286 (+7.1)*	0.273 (7.1)*
0.50 mg/m <sup>3</sup>	0.643 (+5.9)	0.632 (+9.3)	0.269	0.264 (3.5)
5.00 mg/m <sup>3</sup>	0.663 (+9.2%)*	0.635 (+9.9)	0.288 (+7.9)*	0.276 (8.2)*

Power of statistical significance as calculated by the testing laboratory testing laboratory (\* 0.05  $\geq$  p  $\geq$  0.01, \*\* 0.01  $\geq$  p  $\geq$  0.001, \*\*\* p  $\geq$  0.001).

The above table shows that the two highest test dose male kidney weights are elevated relative to the control group. This conclusion is partially based on the consistency of weights obtained for the other three groups. A less convincing case is made for the female high-dose test group because all dosed rats have higher absolute weights than the control group.

TB could not confirm the statistical significance as determined by the testing laboratory because the individual animal data were not presented.

The absolute and relative kidney weights for the rats in the recovery phase of the experiment did not indicate a meaningful difference between the control and dosed group as shown in the following table:

	Absolute Weight (gm)		Relative Weight	
<u>Males</u>	Control	Test	Control	Test
Kidney (right)	1.112	1.153 (+3.7%)	0.276	0.289 (+4.7%)
Kidney (left)	1.117	1.100 (-1.5%)	0.281	0.285 (+1.4%)
<u>Females</u>	Control	Test	Control	Test
Kidney (right)	0.662	0.721 (+8.9%)	0.278	0.276 (----)
Kidney (left)	0.647	0.687 (+6.1%)	0.275	0.272 (-1.5%)

2. Liver. The absolute weight of the male livers was unaffected but the relative weights of the male livers were 6.9 percent higher for the high-dose test group. The absolute weights of the female livers were 12.2 percent higher for the high dose group and this group was also 11 percent higher when relative liver weights were assessed. There were no differences noted between the absolute and relative liver weights after the recovery phase. It should be noted that an increase in liver weight might be expected to be associated with the increase in cytochrome P450 activity.

Other organ weight changes did not demonstrate convincing patterns of test chemical related effects.

The NOEL for this aspect of the study (organ weights) is set at 0.10 mg/m<sup>3</sup>. The LOEL is 0.5 mg/m<sup>3</sup>, at this level kidney (males) weight is affected and at 5 mg/m<sup>3</sup> kidney weight (both sexes) and liver weight are affected.

K. Gross Pathology. Only a limited number of macroscopic findings were reported and these were mostly in the lung and thymus but there were no indications that there were increased incidences with increased dose levels of lindane. The only incidences of macroscopic lesions in the kidneys included "light regions (solid consistency)" and "fluid in the kidneys". These were single isolated incidences.

L. Histopathology. The study report asserts that "no test-substance-related effect could be detected histologically in this lindane inhalation study". Data were presented for the histopathological examination of 34 organs/tissue types. The

only organ deemed to be affected by TB was the kidney and only the kidneys of males were affected. The following table illustrates the findings.

Lesion	Group				
	1	2	3	4	5
Cloudy swelling of the tubule epithelia	2*	-	3	7	8
Dilated renal tubules with protein containing contents	-	-	-	3	3
Proliferated tubules	-	-	-	2	1
Total Examined	12	12	12	12	11 <sup>2</sup>
Total Affected <sup>1</sup>	2	0	3	10	9
% Affected	17%	0	25%	83%	82%

\*Number of incidences.

<sup>1</sup>/As per discussion with Dr. L. Kasza, TB pathologist, these lesions are additive. Since some of the rats had both types of lesions present the number of rats affected is less than the total number of lesions.

<sup>2</sup>/Only 11 rats in this group could be accounted for by this TB reviewer.

Among the rats in the recovery phase, only two controls and a single high-dose group had the lesion described as "cloudy swelling of the tubule epithelia." The other two lesion types in the table above were not found in the recovered animals.

TB concludes that the kidney shows responses to the test material and the NOEL is set at 0.1 mg/m<sup>3</sup>. Note: Although the high dose group females also showed increased kidney weight there was no evidence of histopathological changes noted in the kidneys of this sex.

None of the other organs showed pathological signs of toxicity or other responses to inhalation of lindane. It should be noted that for some organs notably the brain and liver only seven rats were examined.

### Conclusion:

This study is classified as CORE MINIMUM. The report indicates that an extensive amount of work was done to assess the subchronic inhalation toxicity of lindane. Individual animal data were not presented to allow for independent statistical analysis of the blood elements or the clinical chemistry data.

TB does not concur with some aspects of the conclusions made in the study report. In addition to the effects noted by the testing laboratory (clinical signs and increases in cytochrome P<sub>450</sub> and slight increases in liver weight), TB has determined that the kidney of males is also affected by inhalation exposure to lindane. A NOEL for this effect is set at 0.1 mg/m<sup>3</sup> (or 0.1 ug/l).

It should be noted that according to Dr. L. Kasza, TB staff pathologist, the lesions of the male kidneys are best described as mild but potentially they can affect function. The study results, however, do not demonstrate any signs of kidney dysfunction. The kidney lesions noted are transient in nature and were not noted in the rats after allowing six weeks for a recovery phase.

TB has interpreted the results of the myelogram data to indicate that lindane exposure at 5 mg/m<sup>3</sup> results in stimulation of blood cell formation and possibly other effects. No NOEL could be established for this effect because only the high dose test group and the controls were assessed.

Note: TB review found several translation errors but managed to correct the error by referring to the original German text. There was some reference to the recovery period being for four weeks instead of six weeks. The six week period seems to be the correct period based on the starting and finishing dates provided. Also some of the pages of this report were barely readable because of reproduction artifacts.

TB does not concur with the conclusions of the study as presented in the study report. The following NOEL and LOEL summary comments reflect the assessment of TB of this study:

Tentative NOEL = 0.1 mg/m<sup>3</sup> (but see below for discussion of myelogram).

LOEL = 0.5 mg/m<sup>3</sup>. Transient histopathological changes in the kidney structure (males) and increased kidney weight (males)\*.

At 5 mg/m<sup>3</sup>. Clinical symptoms in "in life" phase including diarrhea and piloerection (males and females), increased liver cytochrome P<sub>450</sub> (males and females), increased liver weights (males and females), increased kidney weights (males and females), transient histopathological changes in the kidney structure (males).

At 5 mg/m<sup>3</sup>. (The NOEL for this effect could not be assigned because only the 5 mg/m<sup>3</sup> test group and the controls were assessed.) Effects in the bone marrow as indicated by myelograms to indicate that lindane resulted in stimulation of the formation of the blood elements (males and females but especially males).

\*It should be noted that according to Dr. Louis Kasza, TB pathologist, the lesions of the male kidneys are best described as mild but potentially they can affect the kidney function. The study results, however, demonstrate no signs of kidney dysfunction. No evidence of kidney dysfunction or lesions were observed after allowing 6 weeks for recovery (no dosing).