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[83-2. "Agouti" -indane/1987]

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DATA EVALUATION REPORT

STUDY TYPE: 83-2. Special study with mice.

MRID NO.: None

TOX CHEM No.: 527
PC No.: 009001

TEST MATERIAL: Lindane (gamma-hexachlorocyclohexane): Obtained from the Hooker Chemicals and Plastics, Niagara Falls, New York.

STUDY NUMBER(S): None (journal article).

SPONSOR: None.

TESTING FACILITY: National Center for Toxicological Research, Jefferson, Arkansas.

TITLE OF REPORT: "Tumorigenic responses to lindane in mice: potentiation by a dominant mutation".

AUTHOR(S): G,L. Wolff, D.W. Roberts, R.L. Morrissey, D.L. Greenman, R.R. Allen, W.L. Campbell, H. Bergman, S. Nesnow and C. H. Firth.

REPORT ISSUED: As published in Carcinogenesis 8(12):1889-1897(1987).

CONCLUSIONS:

Evidence for positive carcinogenic response in the agouti and pseudoagouti mouse lines for both liver tumors (hepatocellular adenoma and carcinoma) and lung (papillary and/or solid) tumors.

Clara cell hyperplasia occurs in the lung in all three lines that is apparently irreversible. Liver weight is increased (agouti > pseudoagouti > black) and monooxygenase activity is increased in all three lines (pseudoagouti > black > agouti).

Dose level tested: 0 and 160 ppm. Species: three mouse lines from the NCTR stocks: "agouti", "pseudoagouti" and black. Females only.

Classification: core-SUPPLEMENTARY

Quality Assurance Statement: Not provided.
Good Laboratory Practice Statement: Not provided.

REVIEW

Experimental Constants:

Test Material:

Chemical: Lindane (gamma hexachlorocyclohexane)
Source: Hooker Chemicals and Plastics, Niagara Falls New York
Batch: Lot 6000-425
Purity: Not stated.
Vehicle: None.

Test System:

Species: Three strains of mice as described below. Only females were used.
Supplier: NCTR Breeding Colony
Age: Three to four weeks old at time of allocation to dose groups.
Weight: Initial body weight not provided.
Feed: Purina Laboratory Chow 5010M

The three strains of mice utilized in this study are described as follows:

"Agouti" (A^{vy}/a): This line has increased susceptibility to formation of strain-specific neoplasms (spontaneous as well as virally or chemically-induced), it has a mottled yellow coat color and adult onset obesity.

"Pseudoagouti" (A^{vy}/a): This has the same genotype as the agouti but differs physiologically (it is lean in appearance) apparently because each line has a different degree of mutation expression.

Black (a/a) (YS x VY): This line has a low spontaneous rate of liver tumor formation.

The hybrid mice were produced in the NCTR colony by mating a/a YS females with A^{vy}/a VY males.

B. STUDY DESIGN:

Only control and groups dosed with 160 ppm of lindane were utilized. The dose level of 160 ppm was selected based on a preliminary study which indicated that 160 ppm was the highest dose level which did not result in deaths in a one month study. It is also the dose level tested in the NCI 1977 study in mice. The following experimental intervals were studied.

Continuous feeding:

6 and 12 months: 48 per mouse line for both control and lindane treated.

18 months: 36 of each line for both control and lindane treated.

24 months: 96 of each line for both control and lindane treated.

Discontinuous feeding: (dosed with 160 pm lindane for 6 months and then fed

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control diet for 6 or 18 months).

12 months: 48 agouti and black for both control and lindane treated.

24 months: 96 agouti and black for both control and lindane treated.

"Index" "used to detect appearance of hepatic neoplasms for determination of start of terminal sacrifice". 24 black mice for 18 or 21 months.

Analytical Chemistry: No details of the analytical report were provided. The experimental section of the paper states that dose levels in the feed were determined by the NCTR Division of Chemistry.

Statistics:

Test	Parameters Evaluated
One way analysis of variance with Bonferroni multiple comparisons.	Body and liver weight.
Fisher's Exact Test with 2 x 2 subtables of tumor prevalence for pair-wise comparisons. "SAS" procedure CHRONIC for trends.	Tumor data.

C. SPECIFIC METHODS AND RESULTS:

1. Observations. No details were provided. No reactions to treatment were reported. No information on survival within the individual groups was reported.

2. Body weight. No data on body weight during the in-life phase of the study were reported. Carcass weight (body weight minus the liver weight) was reported as not being affects by lindane treatment (no data were provided). The agouti mice were also stated as being 50 to 100% greater in weight than the pseudoagouti and black mice. The pseudoagouti mice were also thought to be consistently heavier than the black mice but no effect of the lindane treatment was implied.

3. Food consumption and compound intake. Some summary data were provided to indicate that the agouti mice consumed about 7 to 10% more feed than the pseudoagouti and black mice meaning that they also ingested more lindane. The lindane intake in terms of mg/kg/day could not be determined since the body weight data were not provided.

4. Ophthalmological examinations. No determinations were

made.

5. Clinical Chemistry and Hematology. No determinations were made except for the special benzo(a)pyrene monooxygenase activity as described as follows.

S-9 fractions of mouse liver obtained from mice from each line that were dosed with lindane for 6 or 12 months were obtained and frozen and shipped to EPA Carcinogenesis and Metabolism Branch RTP, North Carolina. The assay was designed to assess a P-448 enzyme and the method was not described but provided in a reference. Each of the three lines of mice induced the enzyme as indicated in Table 1.

Table 1. Induction of benzo(a)pyrene monooxygenase (P-448) by lindane in three lines of female mice.

	Mouse Line[1]		
Interval	Agouti	Pseudoagouti	Black
6 months	1.84	2.71	2.07
12 months	1.61	2.78	2.09

1. Data are ratio of treated activity to the control activity. The control activity differed slightly for each line.

These data indicated that lindane treatment at both time intervals induced the lowest increase in p-449 in the agouti line and the most increase int eh pseudoagouti line. The black mouse line was probably closer to the agouti line in its susceptibility to induction by lindane.

6. Urinalysis. No urinalysis data were provided.

7. Sacrifice and Pathology: The mice were sacrificed at their scheduled interval by CO_2 asphyxiation. The report states that approximately 35 tissues were necropsied fixed and trimmed for microscopy. The tissues were dehydrated in ethanol, cleared in xylene, embedded in paraffin, sectioned and stained with H&E. Although additional tissues were apparently prepared, only data on the liver and lung were presented in the report.

8. Organ weight: Data on liver weight following 6, 12 18 and 24 months of treatment were presented. The following summarizes the weight differences.

Agouti. Liver weight was increased 22.4%, 31.2%, 14.7% and 30.8% (all $p < 0.05$) for the 6, 12, 18 and 24 month intervals.

Pseudoagouti. Liver weight was increased 13.5%, 20.3%, 22% and 17.4% (all $p < 0.05$) for the 6, 12, 18 and 24 month intervals.

Black. Liver weight was statistically significantly increased at 24 months only (16.4%). Non significant elevations of 12.2%, 12.2% and 15.4% were also noted.

One aspect of these data is that although the pseudo-agouti line indicated the highest level of P-488 induction, this line did not have a larger increase in liver weight as compared to the agouti line.

Data were also presented that indicated that discontinuation of dosing following six months of dosing with lindane resulted in liver weights equivalent to the controls not dosed with lindane.

9. Histopathology-Individual organ discussions.

A. Liver. Liver lesions were classified according to Firth and Ward. In addition, tumors classified as adenomas corresponded to Types I and II and those classified as carcinomas corresponded to types II and IV according to Becker. The occurrence of liver tumors at the various sacrifice intervals is described below.

6 months. No liver tumors reported.

12 months. 2/48 (4%) control and 3/48 (6%) treated agouti mice developed adenomas. No carcinomas were reported. No tumors were reported in the other lines.

18 months. None (0/34) of the control but 33% (12/36) of the lindane treated agouti mice developed adenomas but there was only one incident of a carcinoma each in the control and treated groups for this line.

In the pseudoagouti line, no liver tumors were reported with lindane treatment although there was 6% (2/35) and 3% (1/34) incidents of adenoma and carcinoma in the pseudoagouti control group.

In the black mouse line, the control group had a higher incident rate (11%, 4/35) for adenomas than the lindane treated group (6%, 2/36). There were no carcinomas at this time interval for this line.

The 18 month data indicate the agouti line to be sensitive to the presence of lindane.

24 months. Table 2 below illustrates the findings for liver tumors following 24 months of continuous treatment with lindane

Table 2. Liver tumors (hepatocellular adenoma and carcinoma) in three lines of female mice dosed continuously with lindane as compared to the control.

	Strain[1]					
	Agouti		Pseudoagouti		Black	
Tumor Type	Control	Lindane	Control	Lindane	Control	Lindane
Number examined	93	94	95	95	96	96
Adenoma	8(9%)	33(35%)	5(5%)	11(12%)	6(6%)	3(3%)
Carcinoma	12(13%)	16(17%)	2(2%)	5(5%)	3(3%)	1(1%)
Total	20(22%)	49(52%)	7(7%)	16(17%)	9(9%)	4(4%)

[1] Data are incidents (incidents as a percentage of number examined).

Black line. There was no indication of increases in tumor incidence in this line of mice as a response to lindane treatment. This line had spontaneous rates of adenomas that were equivalent to the control spontaneous rates for the agouti and pseudoagouti lines. The spontaneous rate of carcinomas was, however, higher (13%) for the agouti line than for either the black (3%) or pseudoagouti (2%).

Agouti line. Clearly adenomas were increased (compare 35% incidence with 9% incidence for the treated and control groups) after 24 months and the increase noted at 18 months above. The incidence of carcinomas in this line were only slightly higher for the treated group (17%) when compared to the controls (13%). Thus, the combined total of tumors is higher but the increase in mostly due to adenomas.

Pseudoagouti. Adenomas appear to be higher after 24 months (compare 12% in the treated group and only 5% in the control group. Similarly carcinomas also appear to be higher although there is only 5% incidence in the treated group and 2% incidence in the controls. The combined incidence is correspondingly higher. There was no indication of an increase in this line at the 18 month sacrifice interval.

Note: The statistical analysis provided in the study report is difficult to interpret. There does not seem to be clear evidence that there was a pair-wise comparison between the control and treated group for each line.

The results of discontinuing the lindane treatment after 6 months and sacrificing the mice either 6 or 18 months later indicated a slight increase in hepatocellular adenomas at 24 months in the treated group (14%. 13/95) when compared to the control (9%. 8/93). There were also 13% (12/93) and 14% (13/95) incidence of hepatocellular carcinomas in the control and lindane treated groups. There were no liver tumors in the lindane

treated mice at the 18 month sacrifice interval.

B. Lung. Compound related increases in Clara cell hyperplasia were noted at the 6, 12, 18 and 24 month intervals. Lung tumors were also increased in the later months of the study for the agouti and pseudoagouti mouse lines. Table 3 illustrates the lung pathology data from this study.

Table 3. Lung pathology data in female agouti, pseudoagouti and black female mice dosed with lindane.

Lung Clara Cell Hyperplasia and Lung Tumors[1]

Interval	Agouti		Pseudoagouti		Black	
	Control	Lindane	Control	Lindane	Control	Lindane
	Hyperplasia					
6	17%	77%	8%	50%	10%	56%
12	31%	92%	17%	76%	14%	90%
18	6%	92%	6%	79%	0	89%
24	15%	72%	10%	76%	10%	82%
	Lung tumors					
6	4%(2/48)	2%(1/48)	2%(1/48)	0	2%(1/48)	0
12	0	2%(1/48)	2%(146)	0	2%(1/48)	0
18	0	17%(6/36)	6%(2/35)	6%(2/34)	0	11%(4/36)
24	4%(4/95)	19%(18/95)	6%(6/95)	14%(13/94)	2%(2/96)	3%(3/96)

1. Data are percentage relative to the number examined. For simplicity the number incidents and number of mice examined is omitted from the hyperplasia table. The lung tumor table presents the percentage and in () the number of incidents/number of mice examined. The same denominator applies for both the non-neoplastic lesion (hyperplasia) and the tumor data for each interval.

An increase in the incidence of Clara cell hyperplasia apparently persisted following discontinuation of lindane dosing after 6 months as indicated by 31% vs 75% for the Agouti line and 14% vs 47% for the black line mice affected for the control and treated groups. After 18 months following discontinuation of lindane dosing, the effect was still apparent with their being 15% vs 42% for the Agouti and 22% vs 10% for the black mouse line.

There was also a slight increase in lung tumors in the Agouti line even though the lindane was discontinued from the diets. For example after 6 months there was 1 incident (2%) in treated group but none in the control. After 18 months, there were 10 incidents (10%) in the treated group but only 4 incidents (4%) in the control group. The lung tumor incidence in the black

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mouse line was equivalent for both the control and lindane treated groups.

In summary, these lung pathology data indicate that there is an increase in the incidence of lung tumors in the Agouti mouse line. This increase in lung tumor incidence may be apparent as early as 18 months in the Agouti line. The pseudo-agouti mouse line also has an apparent treatment related increase but to a lesser magnitude than the Agouti and is only apparent after 24 months. An apparent increase in the incidence of lung tumors in the black line at 18 months was not confirmed at 24 months in a group that had nearly three times as many animals[1].

All three lines are apparently susceptible to lindane dependent increased Clara cell hyperplasia and this condition is apparently irreversible since it was still present even after 18 months after the last lindane dosing.

9. Adequacy of dosing. Since there were no behavioral reactions, in life phase body weight determinations or survival data presented, there is no reliable way to assess if the dose levels were adequate or excessive. Since there was liver weight change, some reviewers may considered that the dose was adequate.

CONCLUSION. The information in this journal article is SUPPLEMENTARY. The data provide a demonstration that the mouse lines (the agouti and pseudoagouti) known to be especially susceptible to spontaneous, viral and/or chemical induction of liver tumors, respond to the dietary presence of lindane by developing more liver tumors. There was no increase in liver tumors in the more standard mouse line (black mouse). The agouti and pseudoagouti mouse lines also had apparent compound related increases in lung tumors. All three mouse lines developed Clara cell hyperplasia in the lungs and this condition is apparently irreversible.

The study is not considered a candidate for upgrading to MINIMUM or GUIDELINE for the following reasons: the protocol included only females, there was only a single dose level tested, only evidence of lung and liver histopathology was presented (no evidence of histopathological examination of the many other tissues was presented), no analytical chemistry report was presented and no individual animal data were presented.

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[1]The mice in the 18 month group were assessed for humoral immune function and the lung tumors were found in this group. The study report suggests that the increase in lung tumors may be somehow related to the immune function testing for the black mouse line.