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EFFECTS OF LINDANE UPON REPRODUCTIVE FUNCTION IN A 3-GENERATION STUDY IN RATS

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SUMMARY

A 3-generation study, involving the feeding of lindane at dietary concentrations of 25, 50 or 100 ppm to CD strain rats, did not reveal any adverse effects upon reproductive function as compared with that of control animals. There were no major malformations, while the distribution of minor variants was not compound- or dose-related. An examination at 21 days of age of 10 ♂♂ and 10 ♀♀ F3B animals in each group revealed a dosage related tendency for increased liver weight and enlarged hepatocytes were seen in some control and treated animals. The relevance of these latter findings was considered of doubtful importance compared with the lack of effects on the growth and reproductive performance of the preceding generations.

INTRODUCTION

The background to the present studies has already been given [1]: they provide details of observations to which reference has been made in the monograph on lindane [2] and in a WHO technical report [3].

METHODS

120 weanling rats of the CD strain obtained from the Charles River Breeding Laboratories, St. Aubin-les-Elbeuf, France, and weighing between

Abbreviations: F0, F1, F2, F3, original, first, second and third generations, addition of A denotes first mating litters, B denotes second mating litters; WHO, World Health Organisation; ♂, ♂♂, male, males; ♀, ♀♀ female, females.

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70 and 80 g were randomly allocated to 4 treatment groups, each comprising 10 ♂♂ and 20 ♀♀. Group 1 acted as controls, while rats in Groups 2, 3 and 4 were given 25, 50 and 100 ppm lindane, respectively, in their diet (Spiller's Laboratory Small Animals Diet) which, with water, was available at all times. Fresh batches of diet were mixed each week, admixture with lindane being by a method similar to that already described [1]. Treatment continued throughout the study for all generations and test animals, the ♂♂ and ♀♀ of each generation, i.e., F0, F1B and F2B (Fig. 1), were maintained on their respective diets for 60 days prior to mating. During this period, parent animals were housed 5 to a cage, with ♂ and ♀ cages alternating. This method was adopted to prevent the hypersensitivity sometimes induced by individual caging, and the possible development of anoestrus when the locale of females is widely separated from that of males.

During the 19-day mating period the rats were housed on the basis of 1 ♂ and 2 ♀♀; the ♂♂ were subsequently returned to their original cages and the ♀♀ were transferred to individual solid floor cages for the birth and rearing of litters. Approximately 10 days after the weaning of first litters the animals were mated a second time, employing alternative ♂ and ♀ pairings. From the resulting second litters, 10 ♂♂ and 20 ♀♀ were selected from each group at

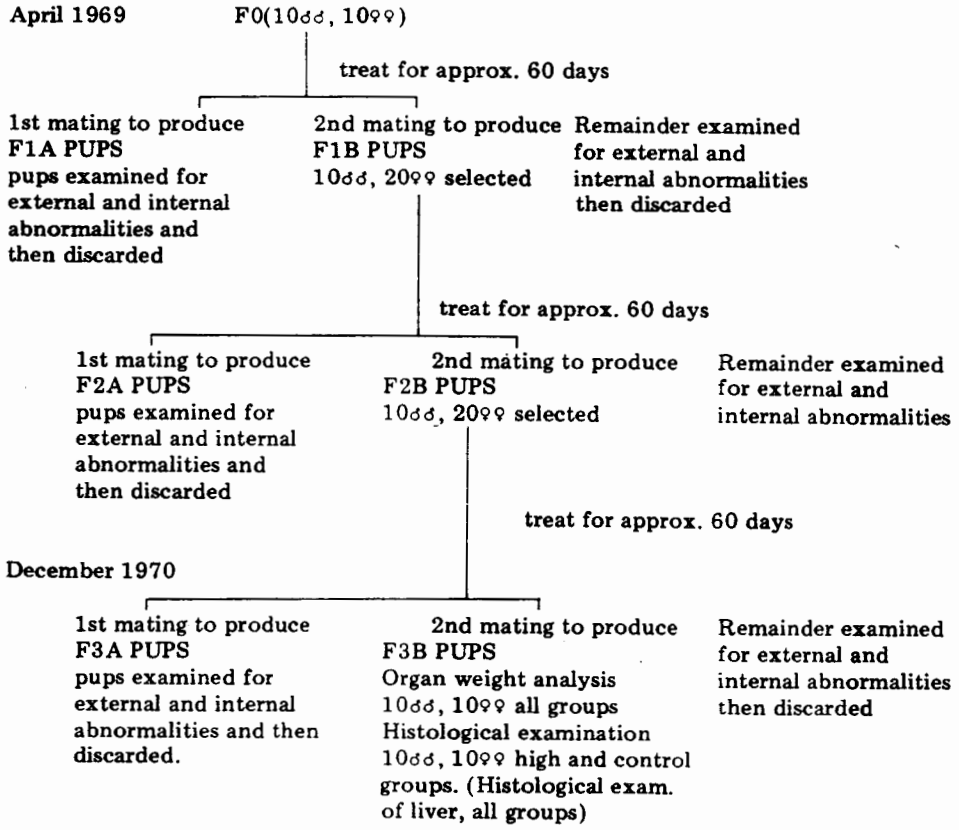

Fig. 1. Experimental design.

weaning to form the basis of the next generation. The animals were selected from as many litters, and as close to the mean weaning weight as possible. The above procedure was repeated twice to permit the histological examination of some F3B pups. Brother and sister matings were avoided for the second and third generations. The chronological sequence of the study is summarised in Fig. 1.

Throughout the study all animals were handled regularly and examined for signs of reaction to treatment. Food consumption was determined weekly during the first pre-mating period of the F0, F1B and F2B generations. Pregnant dams of the F0 generation and the first mating of the F1B generation were weighed weekly from day 0 of pregnancy to the end of lactation. At the second mating of the F1B generation and both matings of the F2B generation pregnant dams were weighed on days 0, 7, 14 and 20 of pregnancy, and 0, 7, 14 and 21 post partum. Pregnancy rate was assessed as the ratio of the number of litters born to the number of ♀♀ paired, expressed as a percentage. Mating performance was judged by taking vaginal smears to determine the duration between initial placing together and coitus; this procedure also indicates whether the oestrus cycle is normal, and may clarify any effects on conception rate. The mating performance and conception rate data enabled the duration of gestation to be determined.

Within 12 h of birth all young were counted, identified by toe amputation, and examined for external abnormalities. The pups were weighed individually at birth, and at 4, 12 (except for the F0 generation) and 21 days post partum. Litter weight was calculated from these individual weights. Keeping nest disturbance to a minimum, all litters were examined daily, up to day 21 post partum, for dead young (which were autopsied unless excessively autolysed or cannibalised) and abnormal young. Pup mortality rates were expressed as absolute group total values and as percentages obtained by first calculating the percentage loss within the individual litters, and then deriving the group mean from the individual litter percentages. At birth, mean values were determined both for the total number of young and for the number of viable and dead young. At 4, 12 and 21 days mean values were calculated in 2 ways: mean A was the number of young to number of litters born, while mean B was the number of young to number of litters with some pups surviving to 21 days. This procedure was adopted because mean A values are affected by the loss of whole litters, which may be due to maternal neglect or to effects upon the parent animal. Mean B excludes such losses, and indicates any general effect on all young.

Young of the first litters (F1A, F2A and F3A generations) and surplus young of the second litters were sacrificed 21 days post partum, sexed, and examined for external and internal abnormalities. Pups with suspected abnormalities were preserved routinely in buffered formalin, or in Bouin's solution if it was considered that free-hand serial sectioning would be of value.

Rats of the F3B generation were killed when 3 weeks old and subjected to detailed internal and external examination for detection of abnormal devel-

opment, as for previous generations. In addition, the brain, liver, heart, pituitary, spleen, thyroid, kidneys, thymus, adrenals, lungs and gonads were removed from 10 ♂ and 10 ♀ animals from all groups and weighed. For assessment of inter-group differences, organ weights were subjected to analysis of co-variance — with final bodyweight as the co-variant — and adjusted values compared where appropriate. Samples of these tissues, together with pancreas, bladder, bone, bone marrow and sections of stomach, small and large intestines were examined microscopically, initially for animals of Groups 1 and 4 only. Subsequently microscopic examination of the liver was extended to include animals in all groups. A further 10 ♂ and 10 ♀ animals from each group were cleared, stained with alizarin, and subjected to skeletal examination.

The lindane content of the diet remained reasonably consistent with the and 12 months, and again at the termination of the study.

RESULTS

The lindane content of the diet remained reasonably consistent with the intended concentrations (Table I).

Parent animals

Treatment was without apparent effect on the parent animals in any generation. Occasional deaths were observed in the F0 and F1B generations, but were considered unrelated to treatment. There were no deaths in the F2B generation.

Over the 3 generations, intergroup variation in bodyweight change did not show any consistent dosage-related trends. For example, among ♂♂ treatment at 100 ppm was associated with a marginally lower weight gain for the F0 generation but not for the F1B and F2B generations. Similarly at 50 ppm, weight gain was slightly greater than that of controls for the F0 and F1B generations, but not for the F2B generation. Among ♀♀ weight gain prior to mating in the test groups (133 g at 25 ppm; 137 g at 50 ppm, 131 g at 100 ppm) was marginally lower than that of controls (145 g) for the F0 generation, but essentially comparable with that of controls for the F1B and

TABLE I

DIETARY CONCENTRATIONS OF LINDANE (ppm)

Theoretical concentration ppm	Observed in samples taken at		
	4 months	12 months	Termination
100	83	124	102
50	56	61	40
25	22	22	23
0	0	0	< 2.5

TABLE II

GROUP MEAN FOOD CONSUMPTION (g/RAT) DURING THE FIRST PREMATING PERIOD

Generation	Duration (weeks)	Control		Lindane 25 ppm		Lindane 50 ppm		Lindane 100 ppm	
		♂	♀	♂	♀	♂	♀	♂	♀
F0	9	1335	1039	1322	1017	1330	1034	1275	1010
F1B	7	1307	998	1244	909	1261	943	1200	941
F2B	7	1272	888	1244	950	1241	965	1247	934

TABLE III

PREGNANCY RATES (%) OVER 3 GENERATIONS

Lindane concentration (ppm)	Generation and Mating						Overall Mean
	F0		F1B		F2B		
	1st	2nd	1st	2nd	1st	2nd	
0	90	80	70	50	90	95	79
25	100	90	95	74	100	75	89
50	95	85	85	65	75	70	79
100	95	95	95	70	85	85	88

TABLE IV

MEAN GESTATION PERIOD (DAYS) OVER 3 GENERATIONS

Lindane concentration (ppm)	Generation and mating					
	F0		F1B		F2B	
	1st	2nd	1st	2nd	1st	2nd
0	21.8	22.0	22.0	21.9	22.1	22.0
25	22.0	22.0	22.5	22.2	22.0	22.2
50	21.9	21.9	22.2	22.3	21.9	21.9
100	22.1	21.3	22.3	22.3	22.2	22.1

F2B generations. No consistent variation was evident in weight changes during pregnancy and lactation.

Intergroup variations in food intake did not show any consistent dosage-related trends, although the F0 generation ♂♂ at 100 ppm ate marginally less food than other groups, while for the F2B generation the average food intake of all test group ♀♀ was slightly higher than that of the controls (Table II).

Minor variations in mating performance and pregnancy rate (Table III)

were unrelated to dosage at any mating, and the duration of gestation was unaffected by treatment (Table IV).

Autopsy of all adults of all generations revealed very few macroscopic changes. Those noted were confined to the F1B generation, and appeared to be unrelated to treatment, i.e., a mammary adenofibroma in a control ♀; a mammary adenoma in a ♀ at 25 ppm; and an area of haemorrhage within the mesometrial adipose tissue in a ♀ at 100 ppm.

Progeny

There were 4 total litter losses among the F0 generation, and one each among the F1B and F2B generations. As the distribution of these losses was unrelated to the dietary concentration of lindane, other comparisons were restricted to those litters containing some young at weaning (mean B values).

Treatment appeared not to have any adverse effect on litter size or on litter and mean pup weights from birth to day 21 post partum. The only differences from control values to attain statistical significance were the greater litter size at 50 ppm ($P < 0.05$, Wilcoxon test) at the first mating of the F2B generation (Table V), and higher litter weights at 50 and 100 ppm ($P < 0.05$) and lower cumulative loss (pup mortality) at 100 ppm ($P < 0.01$) at the second mating of the F2B generation (Table VI).

TABLE V

GROUP MEAN LITTER DATA 1st MATING

Lindane concentration ppm	No. of litters	At Birth				At 21 days post partum			
		Litter size		Litter weight (g)	Mean pup wt. (g)	Litter size	Litter wt (g)	Mean pup wt. (g)	Cumulative loss %
		Total	Viable						
F0 Generation									
0	18	11.2	11.2	70.9	6.5	10.6	605.3	59.6	6.1
25	19[a]	10.7	10.5	67.1	6.6	10.4	556.6	55.6	2.3
50	19	10.3	9.9	62.0	6.5	9.4	511.8	58.7	9.2
100	20	11.5	11.4	71.5	6.3	11.0	597.3	54.7	4.9
F1B Generation									
0	14	11.4	11.4	70.0	6.4	10.0	488.0	51.4	10.4
25	18[a]	10.7	10.3	65.1	6.5	9.6	496.2	53.1	8.8
50	17	12.3	12.2	75.8	6.3	11.2	556.6	50.5	7.6
100	19	10.8	10.6	67.1	6.5	10.1	520.4	53.8	8.7
F2B Generation									
0	18	11.7	11.5	72.4	6.3	11.0	564.0	51.7	5.6
25	20	12.4	12.0	73.0	6.1	11.7	562.2	48.8	5.8
50	15	13.8*	13.4*	80.4	6.0	12.8*	609.0	48.4	6.8
100	17	12.3	12.1	71.8	6.0	11.3	581.9	52.4	7.9

Cumulative loss, pup mortality; total litter losses excluded: a = 1; difference from control statistically significant at Wilcoxon test: *$P < 0.05$.

TABLE VI

GROUP MEAN LITTER DATA, 2nd MATING

Lindane concentration ppm	No. of litters	At Birth				At 21 days post partum			
		Litter size		Litter weight (g)	Mean pup wt. (g)	Litter size	Litter wt (g)	Mean pup wt (g)	Cumulative loss %
		Total	Viable						
F0 Generation									
0	16	11.9	11.8	73.3	6.4	10.6	548.7	53.1	10.1
25	16[b]	13.4	12.9	80.6	6.3	11.2	553.9	50.3	16.4
50	17	11.4	11.0	72.0	6.7	9.7	510.0	51.5	13.5
100	18[a]	12.2	11.9	73.4	6.2	10.5	533.1	51.4	13.9
F1B Generation									
0	10	10.1	10.0	63.4	6.5	9.7	520.4	55.4	5.3
25	14	11.8	10.9	71.7	6.7	10.5	555.9	54.0	10.7
50	13	11.6	11.1	68.5	6.4	10.2	551.9	57.2	12.1
100	14	11.3	10.7	69.6	6.5	10.4	554.2	54.2	8.1
F2B Generation									
0	19	10.7	10.1	61.7	6.3	8.8	424.4	49.5	18.1
25	15	10.3	10.1	64.9	6.5	9.1	450.5	50.8	10.2
50	14	11.6	11.4	71.0*	6.3	10.8	502.9*	47.0	7.2
100	16[a]	10.4	10.2	67.6	6.7	10.1	521.7*	52.0	3.5**

Cumulative loss, pup mortality; Total litter losses excluded: a = 1, b = 2; Difference from control statistically significant at Wilcoxon test: * $P < 0.05$; ** $P < 0.01$.

Only 2 major malformations were observed. 1 rat in the F1B generation, Group 3, failed to mate and autopsy revealed a non-patent vagina. One pup in the F3B generation, Group 4, showed cerebellar hypoplasia. There was no evidence that the incidence of malformations was affected by treatment.

Macroscopic examination of organs of the F3B generation revealed only minor changes, unrelated to treatment.

Organ weights were significantly correlated with body weight (co-variance analysis) for all organs except the pituitary and — with the exception of this organ — all inter-group comparisons, therefore, were made on adjusted (for body weight) weights. For the pituitary, inter-group differences in absolute weight were compared. The only significant differences with an apparent relationship to treatment were the significantly higher liver weight of ♂♂ at 100 ppm and ♀♀ at all dietary concentrations of lindane (Table VII).

Preliminary histological examination of 10 ♂♂ and 10 ♀♀ from each group of the F3B generation suggested that enlarged hepatocytes were seen more frequently in livers of animals in the 50 ppm and 100 ppm groups than in the control and 25 ppm groups, as indicated in Table VIII. The lobular pattern was unaltered, but margination or vacuolation of the cytoplasm was noted, as well as enlargement of the hepatocytes. Inter-group differences were not statistically significant ($P > 0.05$, Exact test, 1-tailed). No other

TABLE VII

GROUP MEAN ORGAN WEIGHTS[a], F3B GENERATION

Lindane concentration (ppm)	Body wt. (g)	Brain (g)	Pituitary (mg)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Thymus (g)	Kidney (g)	Thyroid (mg)	Adrenals (mg)	Gonads (g)
MALES												
0	49.5	1.408	3.40	0.314	0.611	2.525	0.207	0.196	0.696	8.13	18.41	0.243
25	51.2	1.378	3.00	0.328	0.795*	2.697	0.217	0.187	0.683	7.34	19.45	0.239
50	48.8	1.403	3.40	0.307	0.698	2.789	0.211	0.170	0.635	6.91	17.88	0.255
100	54.2	1.448	2.90	0.341	0.556	3.129***	0.196	0.177	0.656	6.62	18.86	0.283
FEMALES												(mg)
0	48.9	1.322	3.40	0.285	0.647	2.422	0.224	0.193	0.679	6.90	16.19	13.17
25	49.7	1.330	2.60	0.320	0.789*	2.726*	0.230	0.180	0.689	6.09	15.69	17.05
50	47.4	1.385	2.10	0.326	0.681	2.740*	0.192	0.169	0.628	6.31	15.78	15.55
100	52.5	1.443**	3.20	0.299	0.553	3.022***	0.185	0.199	0.673	7.10	19.14	16.83

[a] All organ weight values except pituitary weight (absolute values) adjusted for bodyweight by covariance analysis.
Difference from control significantly different at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE VIII

INCIDENCE OF PROGENY WITH ENLARGED HEPATOCYTES OBSERVED IN THE F3B PROGENY SUBJECTED TO HISTOLOGICAL EXAMINATION

Sex	Control	Lindane		
		25 ppm	50 ppm	100 ppm
Male	1/10	2/10	4/10	5/10
Female	3/10	1/10	5/10	4/10

TABLE IX

INCIDENCE OF SKELETAL VARIANTS AT 21 DAYS POST PARTUM (F3B PROGENY)

Lindane level (ppm)	Number of young											
	Examined		With misshapen 5th sternebra		With 7 sternebrae		With bipartite thoracic centrum		With 14th rib(s)		Affected[a]	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	10	10	—	—	4	1	—	—	—	—	4	1
25	10	10	—	—	2	1	1	1	—	1	2	2
50	10	10	1	—	—	1	4	2	1	—	4	3
100	10	10	1	—	—	2	—	—	3	1	3	3

[a] Pups with more than one variant are included only once in the total affected, although they may be entered in more than one of the preceding columns.

histopathological change or variation from normal was seen that could be considered to be associated with administration of lindane.

Major skeletal malformations were not observed in any of the F3B young examined, and the distribution of minor variants did not show a consistent relationship to dosage (Table IX).

DISCUSSION

This study was undertaken primarily to assess the effect of lindane upon reproductive performance of 3 generations of rats following the administration of the compound in the diet at concentrations up to and including 100 ppm. An overall assessment of the results obtained together with the negative results of teratogenicity and mutagenicity studies [2,6] are not considered to provide evidence for a deleterious effect of lindane upon reproductive performance.

Histological examination of the major organs of the offspring was con-

fined to a small sample of the F3B young in accordance with generally accepted practice. This practice is subject to criticism for the low number of animals taken and for the ensuing difficulties in assessing the significance of any histological changes that might be observed [7]. Thus in the present study it was difficult to determine the relevance of the increase in liver weight and the less consistent increase in the incidence of F3B pups showing enlarged hepatocytes. However, considering the successful growth and reproductive performance of the progenitors of the weanlings examined, the liver changes observed in the 21-day-old offspring would appear to be of doubtful biological importance.

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